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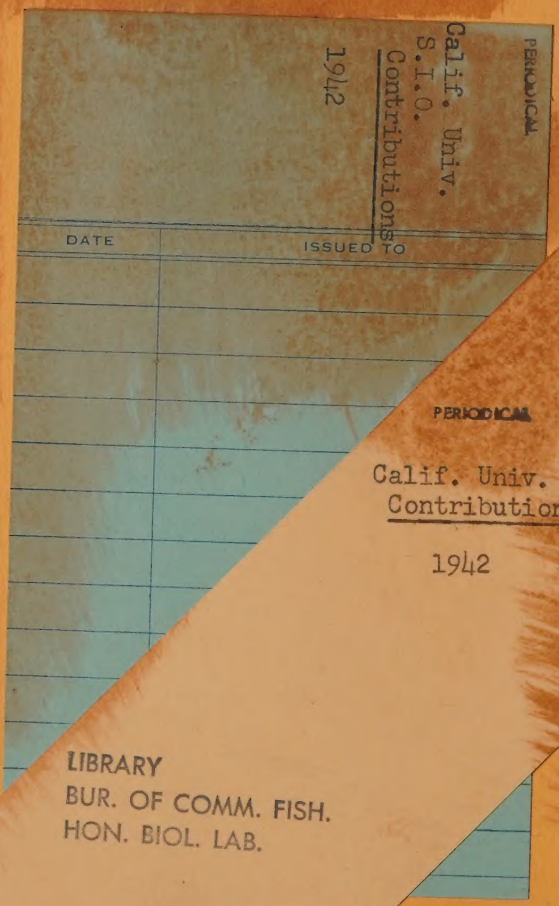

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This volume contains contributions from the Scripps Institution of Oceanography which appeared during 1942 in publications other than *Bulletins of the Scripps Institution of Oceanography* and *Records of Observations of the Scripps Institution of Oceanography.*

LA JOLLA, CALIFORNIA

March, 1943

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Contribution No. 172

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THE BACTERIAL FLORA OF A MARINE MUD FLAT AS AN ECOLOGICAL FACTOR [1]

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Bacteria are generally regarded merely as the causative agents of disease or at most as biochemical agents which play a role in the carbon and nitrogen cycles in nature. A few highly specialized ecologists have studied the factors which influence the distribution of bacteria and allied microorganisms in various environments but relatively little attention has been devoted to a consideration of the importance of bacteria as an ecological factor which influences the distribution of plants and animals. However, the fragmentary observations which have been made indicate that bacteria and allied microorganisms influence the distribution and activity of other plants and animals in aquatic, soil or mud environments just as do temperature, nutrient concentration, salinity, oxygen tension, hydrogen-ion concentration, or other factors which are usually stressed by ecologists.

Bacteria are almost universally associated with plants and animals wherever the latter happen to be growing, with the exception of a few of the lower organisms which by rigorous procedures have been maintained free of bacteria in the laboratory. In soil and water they usually outnumber protozoa, algae, fungi, or any other form of life a hundred or more to one. Due to their minute size bacteria may be regarded as insignificant but by virtue of their large numbers, rapid rate of multiplication, and intense biochemical activity they have a pronounced effect upon physico-chemical, chemical, and biological conditions. This has been demonstrated experimentally in the laboratory and by field observations.

A marine mud flat offers excellent opportunities for such an ecological study and supplies material for controlled laboratory experiments. As pointed out by MacGinitie ('35) a mud flat may be considered as a specialized portion of the ocean floor conveniently located for study. The exposed flat is fairly permanent and most of the inhabitants are sessile and fairly long-lived in contradistinction to circulating water with its mobile population.

Field and Laboratory Studies

Mud flats in Mission (or False) Bay have been under observation since 1933. Large numbers of burrowing worms, clams, mussels, snails, slugs, whelks, limpets, crabs, sea-anemones, protozoa and several other kinds of animals dwell in the mud (Johnson and Snook, '27). Other animals and also plants live primarily at or on the surfaces of the mud, and the water itself supports an extensive population. The Bay (fig. 1) is a shallow inlet of the ocean located midway between San Diego and La Jolla, California. At high tide it occupies an area of four square miles and at low tide it is reduced to channels and lagoons of less than half this area. About three square miles of mud flats are exposed at neap tide. For the most part mud flats were selected for study which were surrounded by water at low tide in preference to those around the edge which are more susceptible to terrestrial contamination. The average depth

[1] Read before the Ecological Society of America at the A. A. A. S. meetings in Pasadena, June 19, 1941. Technical assistance in these studies was furnished by the personnel of the Works Projects Administration, Official Project No. 665-07-3-141. Sustained interest has been inspired by Prof. G. E. MacGinitie, who has given much encouragement and helpful advice. Our chief collaborators have been George G. Ford and Charles Teter whose contributions are gratefully acknowledged. Contribution from the Scripps Institution of Oceanography, New Series No. 152.

FIG. 1. Mission Bay, a shallow inlet of the ocean between San Diego and La Jolla, California. Heavily shaded areas are exposed at mean sea level and stippled areas enclosed by dotted lines are exposed when the water is 1.0 foot below mean sea level. These latter areas which are exposed for a few minutes up to three or four hours every day of the year were the ones investigated. They are flooded with water twice a day, sometimes to a depth exceeding five feet.

of the Bay is only one meter, so a considerable portion of water is exchanged at each tidal cycle. This together with the fact that there is no fresh water dilution except during the brief rainy season, tends to keep the salinity of the water very similar to that in the ocean (Moberg and Allen, '27). However, there are pronounced changes in other characteristics of the water which covers the mud flats. These may be attributed primarily to bacterial and other biological activity, fluctuations in temperature, and exposure of shallow layers of water to the atmosphere.

The only sewage which is discharged into the Bay is that from a small meat packing plant near highway 101. There is no appreciable drainage from San Diego River into the Bay except following prolonged periods of precipitation during the rainy season in the winter months, since most of the water is impounded in a series of reservoirs. Even during 1940–41 when the rainfall exceeded the normal by over 100 per cent, the salinity of the water flowing out of Mission Bay was reduced only from a normal of 3.36 per cent to 2.98 per cent at the lowest. However, following some of the storms the water in the upper part of the Bay, and particularly that in the vicinity of the mouth of the river, the water was virtually fresh

for short periods of time at neap tide, thereby injuring many of the salt marsh plants and animals.

Corroborating observations of Moberg and Allen ('27), it was found that the temperature of the outflowing water may be two or three degrees centigrade higher than that of the inflowing water at the narrow mouth of the Bay during the summer months when the air temperatures are higher than the water temperatures. The temperature of the water in localized areas over the mud flats is sometimes four or five degrees higher than that of the inflowing water from the ocean. The outflowing water is rarely colder, and then only slightly colder, than the inflowing water during the winter season.

The hydrogen-ion concentration of the water over the mud flats is higher than that of the inflowing oceanic water.

TABLE I. *Bacterial content of inflowing and outflowing water of Mission Bay*

Date	Inflowing water	Outflowing water
	Bacteria per cc.	*Bacteria per cc.*
3/23/25	2,100	67,000
3/24/35	870	102,000
3/25/35	2,900	143,000
7/22/35	1,360	380,000
7/23/35	430	96,000
2/16/36	7,200	2,060,000
2/27/36	1,740	1,850,000
Average of 34 days	1,472	294,800
Minimum	194	31,000
Maximum	9,600	4,400,000

While this may be due partly to the exposure of shallow layers of water to the atmosphere, it is attributed primarily to the production of acidic substances by bacteria and other organisms as will be elaborated below. Similarly the oxygen tension of the water over the mud flats is usually decreased in spite of its contact with the atmosphere, another change which is influenced by bacterial activity.

Invariably the bacterial population of the water over the mud flats was higher than in the inflowing oceanic waters. This is illustrated by the data in table I. The water samples were collected on the boulevard bridge at the mouth of the Bay. The bacteria were enumerated by standard plate count procedures using the nutrient media described by ZoBell ('41). It is beyond the scope of this paper to discuss the factors which influence the fluctuating abundance of bacteria in water, the point being that the bacterial population of the water is increased by mud flat conditions. Incidentally, daily analyses of samples of water collected from the end of the S.I.O. pier which extends 1000 feet into the ocean showed that the bacterial population of ocean water ranged from 21 to 30,700 per cc. since 1932, the average being 834 per cc. From the data in table I it will be observed that the Bay water contained from a hundred to a thousand times as many bacteria as water in the open ocean nearby.

The mud itself contains many more bacteria than the overlying water. Whereas there were thousands of bacteria per cc. of water, there were millions of bacteria in an equivalent volume of mud. Bacteria were found in the mud at depths far below the regions where other types of organisms occurred. Samples for bacteriological analysis were obtained by forcing a brass tube 1½ inches in diameter into the mud. In later work the brass tube was lined with a snugly fitting glass tube or with a celluloid cylinder. This latter procedure facilitated the observation of the mud cores thus obtained. Central portions of the mud cores from different depths were dissected out aseptically, weighed into tared bottles, diluted with sterile sea water, and plated on nutrient media. While this procedure detects only a small fraction of the bacteria present as indicated by direct microscopic counts (Bere, '33), it yields comparable results, and it supplies cultures which can be isolated for further study.

Table II shows the number of bacteria which were found in different strata of three mud cores collected at low tide from a mud flat all within a radius of a meter. It will be observed that although the bac-

TABLE II. *Number of bacteria per gram of mud (dry basis) found at different depths of mud from Mission Bay*

Core depth in cm.	Core No. 17	Core No. 18	Core No. 19
0– 5	460,000,000	172,000,000	196,000,000
5–10	9,000,000	43,000,000	21,000,000
15–20	2,710,000	29,000,000	7,400,000
25–30	890,000	1,060,000	580,000
35–40	640,000	270,000	87,000
45–50	207,000	83,000	152,000
65–70	94,000	117,000	35,000
85–90	62,000	21,000	8,200

terial population is extremely variable, it is high at the surface and decreases with depth. This is characteristic of the vertical distribution of bacteria in the Bay mud as well as that from the ocean floor (Lloyd, '31, ZoBell and Anderson, '36*a*, Rittenberg, '40) except that as a rule the mud flat contains more bacteria than oceanic mud. The abundance and biochemical versatility of the bacterial flora of mud is shown by the work of Williams and McCoy ('35), Kusnetzow ('35), Düggeli ('36), Issatchenko ('38) and others.

Bacteria were found in the mud at depths exceeding four meters but most of the samples which were collected for analysis were from the topmost 25 cm. of mud. The latter is the zone of maximum bacterial activity and it is also the principal biotic zone. In fact, it is estimated that the mass of other organisms in the topmost 10 or 12 cm. of mud is as great as the mass of all organisms found below this depth, and certainly the number exclusive of bacteria and photosynthetic organisms is greater (cf. MacGinitie, '35). The mud itself is of terrestrial origin, primarily silt and sand with some clay, although it is streaked with the calcareous shells of mollusks and the siliceous frustules of diatoms. Large shells often interfere with the collection of core samples.

Anaerobic bacteria were enumerated in oval tubes (Rittenberg et al., '37). The vertical distribution of anaerobes as well as aerobes is given in figure 2. The close

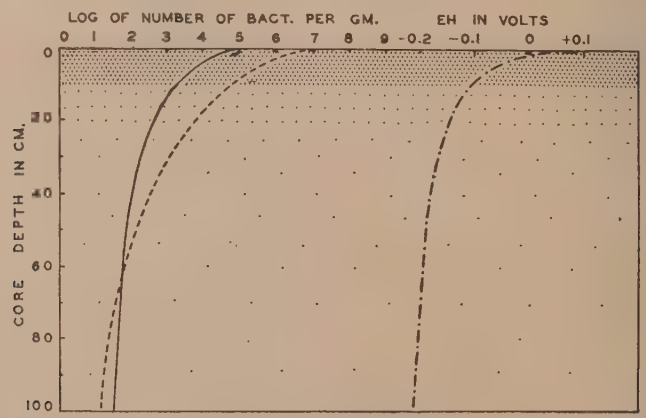

FIG. 2. Average number of anaerobic bacteria per gram of Mission Bay mud (wet basis) found at different depths is shown by the solid line. The broken line shows the number of aerobic bacteria at different depths. The dot-dashed line on the right shows the oxidation-reduction potential of the mud at different depths expressed as Eh in volts. The chart is stippled to indicate the vertical distribution of animals in the mud, there being five points for each animal per cubic foot of mud which was large enough to be retained in a sieve having holes 5 mm. in diameter. It is estimated that there were five times as many smaller animals which passed through the sieve.

correspondence of the number of bacteria in mud from greater depths which developed anaerobically with the number which developed aerobically suggests that the majority of them have the faculty of growing either in the presence or in the absence of free oxygen. Credence is lent to this assumption by the fact that conditions below the topmost few centimeters of mud are anaerobic and also by observations on several isolated pure cultures which were found to multiply in either the presence or absence of free oxygen.

Many of the cultures which were isolated from oval tubes were found to be strict anaerobes. Strict aerobes were rarely found below the topmost few centimeters of mud. This is not surprising in view of the oxidation-reduction potential of the mud, but it was surprising to find several kinds of worms and other burrowing animals at depths exceeding 40 cm. Not only is there no detectable oxygen in the interstitial water at such depths; there is actually an oxygen deficit as indicated by the frequent presence of hydrogen sulphide and by the oxygen-absorbing capacity of the mud when fresh samples were mixed with oxygen-saturated water in glass-stoppered bottles. The oxidation-reduction potential or Eh of mud from different depths is also given in figure 2. The Eh was determined with a platinum electrode and a calomel half cell (ZoBell and Rittenberg, '37) using a Beckman meter.

Reducing conditions usually prevail below the topmost few centimeters of mud, and at depths exceeding 20 or 30 cm. conditions more reducing than Eh —300 millivolts were not uncommon. This, together with the presence of hydrogen sulphide which is often found in a reducing environment, is believed to be the principal factor which limits the depth to which forms of life other than bacteria are found. Not many organisms besides bacteria can live in a highly reducing environment in the absence of free oxygen. Some can make temporary excursions into such environments, others burrow deep into the mud but maintain contact with the oxygenated water above through specialized siphons or tubes, some survive reducing conditions for extended periods of time in a dormant stage, and a few, such as certain protozoa, for example, live normally in the absence of free oxygen (Juday, '19, Clark, '24). As a rule, though, most organisms avoid a reducing environment. The absence of certain animals in black mud of high organic content reported by Rees ('40) is probably due to neither the blackness nor its organic content directly but rather to the reducing conditions. According to Copenhagen ('34) reducing conditions may result in the actual death of animals on the sea bottom and sometimes in the overlying water.

Bacterial activity is believed to be responsible for the reducing conditions in the mud. When placed in battery jars in the laboratory and sterilized, the mud slowly becomes oxidizing in character but upon reinoculation with bacteria it becomes reducing again. Anaerobic bacteria thrive when the oxidation-reduction potential is more reducing than Eh —400 millivolts and, indeed, controlled laboratory experiments have shown that when the mud is mixed with organic matter the bacteria therein can create conditions as reducing as Eh —580 millivolts (ZoBell, '39). Similar reducing conditions in lake mud caused by bacterial activity have been reported by Kusnetzow ('35).

Oxygen Consumption by Bacteria

Bacteria also compete with other organisms for oxygen which may be present in the surface layers of mud. It has been found (ZoBell, '40*b*) that aquatic bacteria consume an average of 14.9×10^{-12} cc. of oxygen per cell per hour in sea water at 22° C., and considerably more than this at higher temperatures or in bay mud which is usually richer in oxidizable organic matter than is sea water. Bacterial respiration is believed to be largely responsible for the decreased oxy-

gen tension of the water over the mud flats and for the lack of oxygen in the mud below the zone where it is used faster than it enters by diffusion from above. Oxygen may be dissolved from the atmosphere, and there is much oxygen produced by diatoms, algae, and other photosynthetic organisms which often occur on the surface of the mud in large numbers. Kusnetzow ('35) considers bacterial activity to be the principal cause of oxygen consumption in lake water.

The oxygen consuming capacity of the microorganisms in the mud is illustrated in table III which shows the amount of oxygen which was consumed by respiring bacteria after different periods of time. One hundred gram portions of surface mud (wet basis) from Mission Bay were weighed into 2500 cc. glass-stoppered bottles and filled with oxygen-saturated sea water. To each bottle was added enough phenol to give 0.1 per cent, a preservative which prevents the multiplication of bacteria without destroying their respiratory enzymes. The oxygen content of the water in some of the bottles was determined immediately by the Winkler method. The other bottles were incubated in a water bath at 22° C. for different periods of time after which the oxygen content was determined. From these data the amount of oxygen consumed by the respiring bacteria was calculated.

TABLE III. *Amount of oxygen consumed by the bacteria in 100 grams of Mission Bay mud after different periods of incubation at 22° C.*

Period of incubation	Sample 145	Sample 155	Sample 165	Sample 175
days	*cc.*	*cc.*	*cc.*	*cc.*
1	0.13	0.04	0.09	0.21
2	0.29	0.17	0.17	0.36
5	0.53	0.31	0.35	0.92
10	0.90	0.51	0.64	1.42
Average per day	0.11	0.06	0.08	0.18

There were enough bacteria and oxidizable organic matter present in 100 grams of mud to consume from 0.06 to 0.18 cc. of oxygen per day over a period of ten days. The gradual decrease in the rate of oxygen consumption is attributed partly to a slow deterioration of the respiratory enzymes and partly to the utilization of the more readily oxidizable fractions of organic matter. While it is dangerous to attempt to translate laboratory findings into terms of what happens in nature, due to a multiplicity of factors which are involved, it is believed that the data in table III at least indicate the order of magnitude of the rate of oxygen utilization by bacteria in 100 grams of mud. At this rate 6 to 18 cc. of oxygen could be consumed per day by the bacteria in a square meter of mud to a depth of 1.0 cm. Thus the oxygen in the mud would be consumed more rapidly than it could be replaced by diffusion, which accounts for the fact that there is no oxygen in the sub-surface layers of mud, and the oxygen tension of the overlying water is reduced.

There are agencies in the mud besides bacteria which consume oxygen, but in the experiments outlined above bacteria are the principal consumers. The rate of oxygen consumption is influenced by the temperature, the concentration and nature of the organic matter content, the kinds of microorganisms present, and several other factors (Miyadi, '34; Waksman and Hotchkiss, '38; ZoBell, '40*a*). In the absence of a preservative such as the phenol used in the foregoing experiment the rate of oxygen consumption is much greater because, as has been pointed out by ZoBell and Anderson ('36*b*), the storage of water or mud samples is accompanied by increased bacterial multiplication. In non-preserved samples from 6 to 30 cc. of oxygen was consumed by 100 grams of mud in 10 days at 22° C.

Hydrogen-ion Concentration

Bacteria have a tendency to alter the hydrogen-ion concentration of their environment. In the presence of appropriate substrata certain bacteria isolated from Mission Bay can increase the hydrogen-

ion concentration to pH 4.5 [2] and others can decrease it to pH 9.5. While they might be responsible for such changes in localized microspheres, the pH range of the mud is much less. The mud is usually somewhat more acid in character than the overlying water, ranging from pH 7.4 to 8.0, the water being from pH 8.0 to 8.3. Although there are several factors which influence the hydrogen-ion concentration, it is believed that the biochemical activities of bacteria are among the most important of the dynamic factors. Some of these activities such as ammonification, sulphate reduction, denitrification, and the utilization of organic acids tend to decrease the hydrogen-ion concentration whereas others such as respiration, fermentation, nitrification, and sulphofication tend to increase it. Bacteria capable of all of these reactions and several others which affect the pH have been found in the mud. Miyadi ('34) has shown that bacterial activity has a pronounced effect upon the hydrogen-ion concentration of lake mud, and Kusnetzow ('35) reports that bacterial activity may affect the hydrogen-ion concentration as well as the oxidation-reduction potential and other physico-chemical properties of an entire water basin.

Bacteria as Food

Perhaps one of the most important roles of bacteria in a mud flat is serving as a source of food for the large and diverse animal population. The mud-dwelling animals probably derive an appreciable amount of their nourishment directly from the ingestion of bacteria. Many protozoa feed upon bacteria (Luck et al., '31), and it has been demonstrated by ZoBell and Feltham ('38) that certain marine invertebrates can live almost indefinitely on an exclusive diet of bacteria. According to MacGinitie ('32) a large proportion of the animals inhabiting mud flats are equipped with specialized feeding mechanisms which enable them to ingest and digest microscopic particles of detritus and bacteria. The bacteria are nutritious, and with rare exceptions are harmless. Baier ('35) and MacGinitie ('35) believe that bacteria constitute an appreciable proportion of the food of mud-eating animals. In the mud there are bacteria present which can live on cellulose, chitin, lignin, and other complex compounds which are unfit as food for most types of animals.

Those animals which are not equipped with specialized filtering mechanisms for catching bacteria ingest them along with detritus to which the bacteria are attached. Not infrequently the mass of bacteria attached to a particle of organic matter or a plankton organism exceeds that of the substrate itself. Baier ('35) opines that most detritus feeders in the mud are nourished by the bacteria which are decomposing the detritus rather than being nourished directly by the detritus upon which they appear to be feeding. This function of bacteria together with the role they play in aiding in the digestion of ingested foods in the intestinal tracts of certain animals may account in part for the failure of biologists to grow the animals in a bacteria-free environment.

Taking 10,000,000 as the average number of living bacteria per cc. of mud gives 2.2×10^{12} bacteria per cubic foot. Since an average bacterium weighs around 0.5×10^{-12} grams, it follows that there are approximately 1.1 grams of living bacteria per cubic foot of mud. The bacteria contain about 90 per cent water but since the plate count procedures used in enumerating them detects a maximum of only 10 per cent of the living bacteria (Bere, '33), there are no less than 1.1 grams dry weight of bacteria per cubic foot of mud. Now cognizance must be taken of the fact that the bacterial population of any given environment at any particular time is merely an expression of the dynamic balance between the rate of destruction and the rate of multiplication of the bacteria. In the laboratory it has

[2] The pH is the reciprocal of the hydrogen-ion concentration denoting the negative logarithm of the concentration of hydrogen-ions in gram atoms per liter.

been ascertained that marine bacteria may multiply more often than once an hour under suitable conditions. Assuming that they divide only ten times a day in the mud, it follows that there would be 11 grams dry weight of bacteria produced per cubic foot per day available for the nutrition of the animal population.

Besides serving as a source of nourishment for animals, bacteria also produce essential plant nutrients thereby contributing to the primary productivity of the water. Waste and dead organic matter are rapidly decomposed by the bacteria, part of it being assimilated directly by the bacteria and part of it being mineralized, leaving ammonia, phosphate, carbon dioxide, sulphate, and other end-products which are used by growing plants. Bacteria which are capable of decomposing nearly all classes of organic matter found in the water or mud have been demonstrated in Mission Bay.

Discussion

The factors which influence the distribution and abundance of bacteria in the mud flat will be discussed elsewhere. While there are marked changes in the quality and quantity of the bacterial population with season, depth, tidal cycles, temperature, organic matter, etc., the quantitative data reported above are representative of mean or average conditions. For present purposes this should suffice to show that bacteria are present in sufficient numbers to play an important role in the distribution and occurrence of the mud flat fauna and flora.

The importance of bacteria as an ecological factor was anticipated by MacGinitie ('35) when he wrote that "The importance of bacteria may be underestimated even in oceanographic investigations but of a certainty they are far more important as a direct source of food on mud bottoms than they have been given credit for in the past. It has long been my contention that bacteria have not been given their just dues as an ecological factor." Fraser ('32), Rees ('40), and Miyadi ('41) refer to the possible importance of bacteria as an ecological factor, and other ecologists often mention the presence of bacteria in "soil associations," but quantitative studies on the effect of bacteria upon physico-chemical conditions and food cycles in the aquatic or soil environment have been neglected by ecologists.

It is believed that detailed observations on the numbers and kinds of bacteria in localized areas may often help to explain the presence or absence of the fauna and flora of these areas. Sudden or abrupt changes especially may be attributed to bacterial activity. This is due to the rapidity with which bacteria may multiply when conditions are favorable and also to the intensity of their biochemical activities. While in general bacteria may be beneficial, serving as a source of food for many animals and by their activities providing plants with a constant rich supply of ammonium, nitrate, and phosphate, they may discourage the presence or growth of plants and animals by competing for oxygen, reducing the oxidation-reduction potential, producing hydrogen sulphide or by markedly changing the hydrogen-ion concentration. Mud flats themselves should be regarded as the site of intense bacterial activity rather than merely being the place where bacteria concentrate by settling from the overlying water.

Summary

The unique topographical features and highly diverse fauna of Mission Bay near San Diego, California, makes it well suited for a detailed study of its bacterial population with particular reference to the effect of bacteria upon physico-chemical and biological conditions.

Over a period of years it has been found that the bacterial population of the water ranges from several thousand to a few million per cc. and the mud contains several million bacteria per gram. Inflowing oceanic water contains only a few hundred bacteria per cc.

The bacterial population of the mud decreases more or less exponentially with depth. Living bacteria are found at depths exceeding five meters where no other kinds of organisms live. By depleting the dissolved oxygen content of the mud, by reducing the oxidation-reduction potential, by producing hydrogen sulphide and by increasing the hydrogen-ion concentration bacteria create environmental conditions at certain places in the mud which are inimical to other forms of life.

Bacteria consume enough oxygen to reduce the oxygen tension of the overlying water, and they increase the hydrogen-ion concentration of the water by 0.02 to 0.3 pH units.

Bacteria are beneficial by serving as an important source of food for mud-dwelling animals and by mineralizing organic matter with the production of plant nutrients.

In view of the different ways in which bacteria influence environmental conditions and the nutrition of mud flat flora and fauna, it is believed that they should be considered as an important ecological factor.

Literature Cited

Baier, C. R. 1935. Studien zur Hydrobakteriologie stehender Binnengewasser. Archiv. f. Hydrobiol. **29**: 183–264.

Bere, Ruby. 1933. Numbers of bacteria in inland lakes of Wisconsin as shown by the direct microscopic method. Int. Rev. d. ges. Hydrobiol. u. Hydrograph **29**: 248–263.

Clark, W. M. 1924. Life without oxygen. Jour. Wash. Acad. Sci. **14**: 123–138.

Copenhagen, W. J. 1934. Occurrence of sulphides in certain areas of the sea bottom on the south African coast. Union So. Africa Fish & Marine Biol. Survey Rept. No. 3. pp. 3–18.

Düggeli, M. 1936. Die Bakterienflora in Schlamm der Rotsee. Rev. d'Hydrol. **7**: 205–363.

Fraser, J. H. 1932. Observations on the fauna and constituents of an estuarine mud in a polluted area. Jour. Marine Biol. Assoc. **18**: 69–85.

Issatchenko, B. L. 1938. Review of the work on the microbiology of mud and mineral springs. Microbiology **7**: 385–410. (Original in Russian.)

Johnson, Myrtle E., and H. J. Snook. 1927. Seashore Animals of the Pacific Coast. Macmillan Co., New York. 659 pp. (1927).

Juday, C. 1919. A fresh water anaerobic ciliate. Biol. Bull. **36**: 92–95.

Kusnetzow, S. I. 1935. Microbiological researches in the study of the oxygen regimen of lakes. Verhandlungen der Internat. Ver. f. Theor. und Angen. Limnologie **7**: 562–582.

Lloyd, B. 1931. Muds of the Clyde Sea area. II. Bacterial content. Jour. Marine Biol. Assoc. **17**: 751–765.

Luck, J. M., G. Sheets and J. O. Thomas. 1931. The role of bacteria in the nutrition of protozoa. Quart. Rev. Biol. **6**: 46–58.

MacGinitie, G. E. 1932. The role of bacteria as food for bottom animals. Science **76**: 490.

——. 1935. Ecological aspects of a California marine estuary. Amer. Midland Naturalist **16**: 629–765.

Miyadi, D. 1934. Oxygen absorption of the lake deposit. Proc. Imper. Acad., Kyoto Imper. Univ. **10**: 236–239.

——. 1941. Marine benthic communities of the Ise-wan and the Mikawa-wan. Memoirs Imp. Mar. Observatory, Kobe **7**: 503–524.

Moberg, E. G., and W. E. Allen. 1927. Effect of tidal changes on physical, chemical and biological conditions in the sea water of the San Diego region. Bull. Scripps Inst. Oceanogr., Tech. Ser. **1**: 1–17.

Rees, C. B. 1940. A preliminary study of the ecology of a mud-flat. Jour. Marine Biol. Assoc. **24**: 185–199.

Rittenberg, S. C. 1940. Bacteriological analysis of some long cores of marine sediments. Jour. Marine Res. **3**: 191–201.

——, **D. Q. Anderson and C. E. ZoBell.** 1937. Studies on the enumeration of marine anaerobic bacteria. Proc. Soc. Exp. Biol. & Med. **35**: 652–653.

Waksman, S. A., and Margaret Hotchkiss. 1938. On the oxidation of organic matter in marine sediments by bacteria. Jour. Marine Res. **1**: 101–118.

Williams, F. T., and Elizabeth McCoy. 1935. The microflora of the mud deposits of Lake Mendota. Jour. Sed. Petrology **5**: 31–36.

ZoBell, C. E. 1939. The occurrence and activity of bacteria in marine sediments. Recent Marine Sediments, Amer. Assoc. Petrol. Geol., Tulsa, Okla. pp. 416–427.

——. 1940a. Some factors which influence oxygen consumption by bacteria in lake water. Biol. Bull. **78**: 388–402.

——. 1940b. The effect of oxygen tension on the rate of oxidation of organic matter in the sea. Jour. Mar. Res. 3: 211–223.

——. 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. Jour. Mar. Res. 4: 42–76.

——, and D. Q. Anderson. 1936a. Vertical distribution of bacteria in marine sediments. Bull. Amer. Assoc. Petrol. Geol. 20: 258–269.

——. 1936b. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull. 71: 324–342.

——, and Catharine B. Feltham. 1938. Bacteria as food for certain marine invertebrates. Jour. Mar. Res. 4: 312–327.

——, and S. C. Rittenberg. 1937. An asbestos KCl bridge and a simple calomel electrode. Science 86: 502.

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153. SURFACE CHEMISTRY OF CAROTENOIDS

1. ASTACENE[1]

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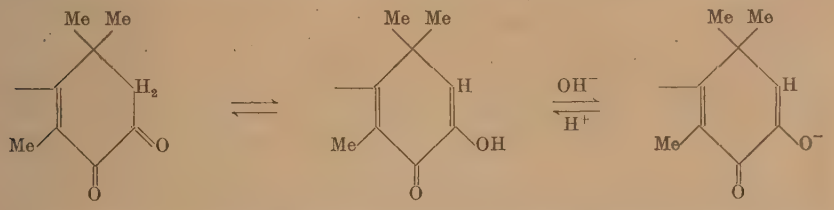
(*Received 24 November 1941*)

Astacene, a carotenoid derived from the naturally occurring pigment astaxanthin [Kuhn & Sorensen, 1938] commonly found in many crustaceans, is a tetraketo-β-carotene [Karrer & Loewe, 1934; Karrer *et al.* 1935] bearing two keto groups on each of the closed ionone rings which terminate a long polyene chain. Tautomeric keto-enol equilibrium conditions render astacene capable of acting as a weak acid, showing reversible formation of salts in the presence of alkali:



The ready hydrolysis of K salts of such carotenoid acids as astacene and metridene, an astacene-like carotenoid from the anemone *Metridium* [Fox & Pantin, 1941], is shared by weak carboxylic acids such as the indicator methyl red. Unless considerable excess base is present in the aqueous phase such salts hydrolyse at the interface between light petroleum and water, and diffuse as the corresponding free acids into the light petroleum layer.

Astacene and metridene are yellow in light petroleum solution but orange-red in pyridine, magenta-red in CS_2 and various yellow, orange or red colours in other organic solvents. Either of these carotenoids, when their light petroleum solutions are shaken vigorously with water, form decidedly pink evanescent oil-in-water emulsions. As these emulsions rapidly break, the pigment diffuses back into the light petroleum layer, again yellow in colour. This reversal of the colour-change with the breaking of the emulsion clearly indicates a difference between the absorption spectra of molecules adsorbed at the light petroleum-water interface and those in bulk light petroleum solution.

The colour-change of astacene may be due to any of several factors:

(1) The effective solvent of astacene at the light petroleum-water interface may differ from pure light petroleum solution. For example, its effective dielectric constant must lie between that of water and that of light petroleum.

[1] Spectroscopic observations by D. L. F.; monolayer measurements by J. F. D.; chromatic emulsions studied jointly.

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Thus the different colour of the interfacial astacene molecules may be due to their being in a different solvent.

(2) If enolic derivatives of astacene are red, such a colour would be produced if the keto-enol equilibrium were shifted in the interface toward the enol form.

(3) If the ion of astacene is red, an increase in ionization of astacene in the interface might be responsible for the shift in colour from yellow to red.

Experimental

Astacene, derived from the astaxanthin of lobster eggs, was recrystallized from pyridine. Surface pressure and surface potential measurements of monolayers were made with techniques described by Adam [1938]. Determinations of absorption maxima were made with the use of a recent model of the Hartridge Reversion Spectroscope [cf. Emerson & Fox, 1940; Fox & Pantin, 1941]. Glass-distilled water was used in all the experiments.

Results: I. With emulsions. Solutions of 20 mg. astacene per l. of light petroleum were shaken with various aqueous solutions. With water, 5*N* HCl, *N*/10 HCl, 5*N* KOH, 5*N* NaOH, *N*/10 NaOH and saturated NaCl solution, the change in colour to pink on shaking was found. Deutsch [1927] has found that colour changes due to ionization of weak acids at interfaces are a function of the *p*H of the aqueous phase. This is not the case with the colour change of astacene, although astacene is a very weak acid. Hence the pink colour cannot be due to ionization; i.e. explanation (3) may be discarded.

When astacene in light petroleum was shaken with 50 % acetic acid, 50 % aqueous pyridine, 50 % methyl or ethyl alcohol, 50 % acetone, 1 % octyl alcohol, 1 % butyric acid or water containing traces of oleic or palmitic acids, the colour change from yellow to pink no longer occurred. While the presence of CS_2 in the light petroleum did not affect the phenomenon, addition of $CHCl_3$ or diethyl ether inhibited it considerably. It also was abolished if traces of cholesterol, lecithin or triolein were added to the light petroleum before shaking with water. The colour change did not vanish abruptly at a particular concentration of an inhibiting substance, but over a range of concentrations of 10-fold to 100-fold. Thus with acetic acid the inhibition of change occurred over the concentration range of 1 % to 10 % in the aqueous phase, with pyridine, between 1 % and 0·01 %, in the aqueous phase; with oleic acid, between 0·1 % and 0·001 %, in the light petroleum phase.

On the other hand, when shaken with Na butyrate, Na oleate, or Na palmitate solution, the pink colour was not suppressed, but possibly even accentuated, and a permanent emulsion formed. The colour change with these soaps appeared only at *p*H values more alkaline than about 11·5. At *p*H 12, a concentration of Na oleate in the aqueous phase 10^4 times greater than the astacene concentration in the light petroleum did not cause inhibition of the colour change. We may provisionally suggest that the persistence of the colour change with soaps is due to formation of a complex between astacene and fatty acid anion; and that, since there is no colour change at *p*H 9 where ionization of fatty acid is almost complete, whereas the change is pronounced at *p*H 12 where the anion of the enol form of astacene probably begins to appear, the complex is possibly formed between fatty acid anion and astacene anion, and not with unionized astacene. This, however, has not been proved.

Summing up the results obtained with emulsions, we may say that all surface-active materials tried, with the exception of soaps, suppress the colour

change. This may reasonably be due to competition of molecules for a position in the petrol-water interface, so that in the presence of sufficient amounts of other surface-active material, astacene molecules are unable to secure a position in the interface, and consequently no colour change occurs. The apparent exception of soaps may be due to formation of a complex, so that in this case astacene molecules are not driven out of the surface; the evidence for the existence of such a complex will be discussed later.

Results: II. Absorption bands in different solvents. It is well known that the absorption bands of carotenoids in the visible region of the spectrum vary from solvent to solvent. Table 1 shows values for the position of the maximum

Table 1. *Position of the absorption band of astacene in the yellow-red zone of the visible spectrum*

Substance	mμ	Colour of solution
Light petroleum	483	Yellow
*cyclo*Hexane	489	Yellow-orange
Acetone	489	Orange
Glacial acetic acid	488	Orange
Benzene	496	Red-orange
Chloroform	497·5	Orange-red
Pyridine	500	Orange-red
Carbon disulphide	512	Red

of the adsorption band of astacene in the region of 490 mμ in various solvents. The changes in colour may involve enolization, but since a similar change is found with other carotenoids which have no polar groups at all, such as β-carotene, it is more probable that the main action of the solvent is on the polyene chain. Table 2 shows the shift in the position of the absorption band

Table 2. *Shift in absorption band on emulsification of light petroleum solutions of carotenoids with aqueous K oleate*

	Absorption band of light petroleum solution		Absorption band of light petroleum solution
	(*a*) Without oleic acid	(*b*) With oleic acid	emulsified with K oleate
Compound	mμ	mμ	mμ
β-Carotene	481	481·5	485*
Zeaxanthin (dihydroxy-β-carotene)	480	482	489·5*
Astacene (tetra-keto-β-carotene)	483	484	493

* First absorption band very diffuse in presence of soap, and difficult to determine accurately; second absorption band, recurring at about 451 mμ obliterated (in Hartridge instrument) in presence of soap.

when a solution of a carotenoid is emulsified in water containing a little K oleate. β-Carotene shows a shift of 3·5 mμ, zeaxanthin of 7·5 mμ, and astacene of 9 mμ. The shifts with the former two substances are not visible to the naked eye, which is probably the main reason why such shifts have not been previously reported.

Thus the shift of absorption band is not confined to astacene, but is also exhibited, though to a smaller degree, by other carotenoids. The values of the shift have the order: carotene < zeaxanthin < astacene. Since these substances have respectively 0, 2 and 4 polar groups, it is to be expected that their tendency to accumulate at an air-water interface will follow the same order, carotene < zeaxanthin < astacene. Hence, if the shift on emulsification is due to the difference between the properties of the interface and bulk phases, it is possible that

the differences in the amount of shift are due simply to the fact that the amounts of the substance actually in the interface follow the series carotene < zeaxanthin < astacene: i.e. the greater shift found with astacene may simply be due to a larger fraction of astacene entering the surface phase on emulsification than is the case with the other substances. Consequently it is not essential, on the evidence at present available, to attribute the larger shift with astacene to enolization. On the other hand, the possibility of enolization being involved cannot be ruled out.

Results: III. With monolayers. Fig. 1 shows surface pressure and surface potential (ΔV) measurements on astacene monolayers spread on 0·01 N HCl. The colour of the monolayers was obvious even at an area of 100 Å.^{2} per molecule.

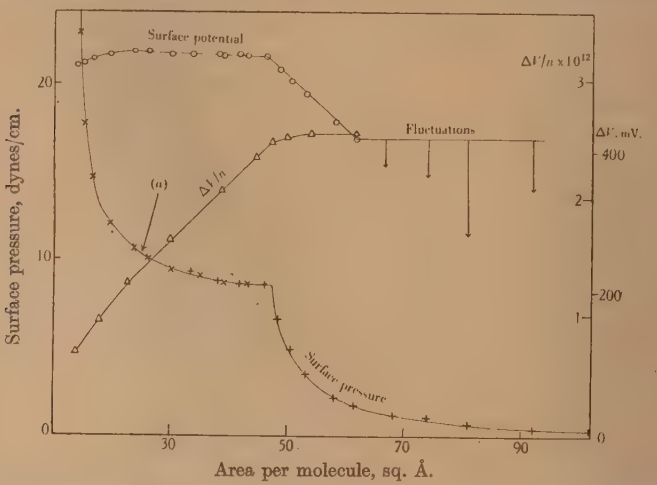

Fig. 1. Surface pressure and surface potential measurements on monolayers of astacene, spread on 0·01 N HCl. The film is solid at areas less than that indicated by (*a*).

At areas greater than 61 ± 2 Å.^{2} the film is electrically heterogeneous, ΔV showing large fluctuations. On compression from 61 Å.^{2} to 47 Å.^{2} the surface pressure rises to 8·9 dynes/cm., the film being electrically homogeneous over this range, ΔV rising from 420 to 530 mV. At 47 ± 1 Å.^{2} there is a discontinuity in both surface pressure and ΔV curves. On compression to areas smaller than 47 Å.^{2} the surface pressure rises slowly, until the film solidifies at 25 ± 1 Å.^{2}, and ΔV rises slowly from 530 to 545 mV. At areas less than 25 Å.^{2} the film is much less compressible, and tends to collapse. Measurements taken rapidly at higher pressures when extrapolated to zero pressure suggest a limiting area of about 18 Å.^{2} for this incompressible part of the film, but this value may be in error, due to collapse. The curve of $\Delta V/n$,* where n is the number of molecules per cm.^{2}, also shows a transition at 47 Å.^{2} Between 47 and 61 Å.^{2}, $\Delta V/n$ is roughly constant. At areas less than 47 Å.^{2}, $\Delta V/n$ falls rapidly as the area per molecule is diminished.

The general behaviour of the films is similar to that of films of other molecules containing two sets of polar groups widely separated by a hydrocarbon chain [Adam & Jessop, 1926; Adam *et al.* 1934] or by a hydrocarbon ring system

* $\Delta V/n$ is a measure of the average contribution per molecule to the observed surface potential.

[Adam *et al.* 1932; Danielli *et al.* 1933]. It therefore seems probable that the characteristics of astacene films are to be explained in the same way as those of, for example, the dibasic esters $CO_2Et.(CH_2)_n.CO_2Et$, or triacetyl oestrin. Thus at areas greater than 47 Å.^{2} per molecule all the polar groups of astacene must be in the water surface, and the transition region between 47 and 25 Å.^{2} is the region in which one set of polar groups of each molecule is removed from the surface, so that at areas less than 25 Å.^{2} the molecules are all standing upright, with one polar group only in the surface, giving an incompressible film. At 47 Å.^{2} the astacene molecule must be bent in a bow, for the area of 47 Å.^{2} is sufficient only for the rings containing the polar groups, and the hydrocarbon chain linking the two rings must be raised above the water surface. This is evidence for considerable flexibility of the polyene chain. The areas of 47 Å.^{2} for two rings in the surface, and of 25 Å.^{2} for the solid film with one ring in the surface, are similar to those given by Adam for simple benzene ring systems. The apparent limiting area of 18 Å.^{2} at zero compression is low for a ring system; as mentioned before, this area may be in error due to collapse.

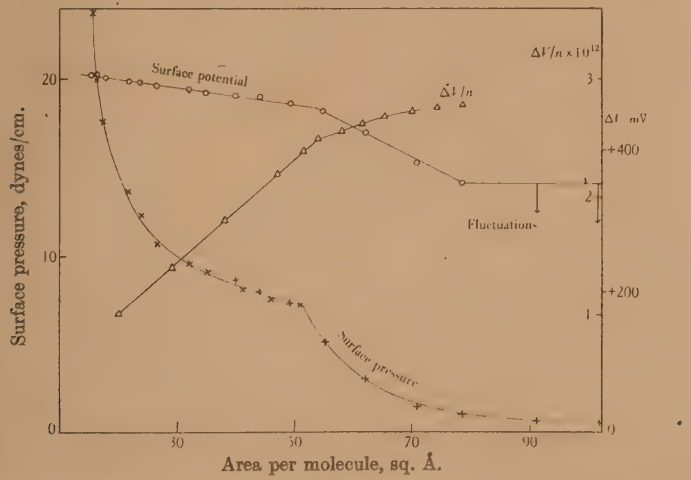

Fig. 2. Surface pressure and surface potential measurements on monolayers of astacene, spread on $N/6$ NaOH.

Fig. 2 shows results for astacene films spread on $N/6$ NaOH. The films are similar in type to those on $N/100$ HCl, but occupy rather larger areas per molecule at a given surface pressure, and ΔV is about 50 mV. lower than on HCl solution. The transition point on the surface pressure, ΔV, and $\Delta V/n$ curves is at 53 ± 2 Å.^{2}

Films were also spread from solutions containing equal numbers of molecules of oleic acid and astacene. Molecules in such films spread on $N/100$ HCl behave in the same way as in films of the separate molecular species, i.e. if it is assumed that in the mixed film oleic acid occupies the same area, and gives the same surface potential at a given surface pressure, as it does in films containing oleic acid only, then the areas and surface potentials calculated for astacene in the mixed film are also the same as in films containing astacene only. The only exception to this is the surface potential, which is greater for astacene in the mixed film than in the film containing astacene only by 70 mV. at an area of

25 Å.^{2} The difference falls to zero at 47 Å.^{2}, within experimental error. This suggests a slight interaction between the dipoles of oleic acid and astacene, occurring only when the latter molecules are standing on end with only one ring system in the interface.

Fig. 3 shows results for oleic acid on $N/6$ NaOH; A for films consisting of oleic acid only; B values calculated for oleic acid from results obtained on films containing equal numbers of molecules of oleic acid and astacene, assuming that in the mixed films astacene occupies an area and gives a surface potential, at a given surface pressure, equal to that given by astacene in films containing astacene only. Oleic acid alone forms a film of gaseous type, with a constant surface potential of -60 mV. In the mixed film, at surface pressures of less

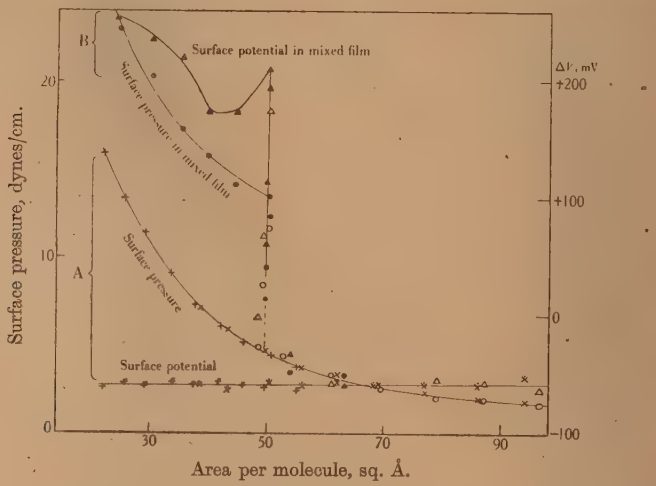

Fig. 3. Surface pressure and surface potential measurements for monolayers of oleic acid spread upon $N/6$ NaOH. Curves A: for films consisting of oleic acid only. Curves B: results calculated for oleic acid from observations made on films containing equal numbers of molecules of oleic acid and of astacene, assuming that the behaviour of astacene molecules on the surface is not changed by the presence of oleic acid molecules.

than 4·5 dynes, the calculated area and surface potential for oleic acid are the same as is found for oleic acid alone. But as the pressure is raised from 4·5 dynes to 13·5 dynes, the calculated area per molecule for oleic acid remains nearly constant, and the surface potential rises from -60 to $+210$ mV. This range of surface pressures is that in which one ring system of each astacene molecule, with its polar groups, is removed from the interface: at the surface pressure of 4·5 dynes, where this change begins, astacene occupies 51 Å.^{2} On further compression of the mixed film the area per molecule calculated for oleic acid falls again, from 50 Å.^{2} at 13·5 dynes to 24 Å.^{2} at 24 dynes, above which surface pressure the mixed film tends to collapse.

From these results the following conclusions may be drawn concerning the mixed film on $N/6$ NaOH: (1) at areas at which astacene has both rings in the interface, there is very little interaction between the oleate ion and astacene; (2) there is a very strong interaction between the oleate ion and astacene molecules oriented with one ring only in the interface and having the polyene chain

roughly perpendicular to the surface. This interaction involves a surface potential increase of +270 mV. and an area increase of 25 Å.^{2} per molecule at a surface pressure of 13·5 dynes, at which the interaction appears to be most marked. It is probable, though not known with certainty, that on $N/6$ NaOH astacene is ionized; so that the interaction is probably between oleate ion and astacene ion, and not between oleate ion and unionized astacene.

This interaction is probably similar to those recently studied by Schulman & Rideal [1937]. Schulman & Stenhagen [1938] and Schulman & Cockbain [1938; 1939] have shown that where a large interaction takes place between two molecular species in an interface, a mixed film of the two species is often more stable than a film of either species alone, and that an initially formed film of one species, when the second species is added to the system, may be converted into the more stable mixed film. The interaction between oleate ion and astacene is very large, and hence the astacene-oleate ion mixed film would be stable at an interface on this view even in the presence of an excess of oleate ion. But the interaction between free oleic acid and astacene is slight and accordingly there is no reason to suppose that a mixed film will be more stable than a film of oleic acid, in the presence of an excess of oleic acid. This may be the reason why free oleic acid can drive astacene out of the oil-water interface, whereas oleate ion, at alkaline *p*H, cannot do so.

Discussion

Of the three possible factors which might cause the absorption spectrum of astacene at an interface between water and light petroleum to be different from the absorption spectrum of astacene in a bulk phase of light petroleum, the third possibility (increased ionization at the interface) has been eliminated. The first factor, that the interface is a different solvent, is probably the major one, since it has been shown that other carotenoids which neither ionize nor enolize show a similar change following absorption. The shift is such that the interface behaves as though it were a more polar solvent than light petroleum but less polar than $CHCl_3$. Adam and Rideal and their colleagues have advanced the view that molecules in monolayers of fatty substances at a water surface will behave as though they were in a solvent of properties intermediate between those of water and a pure hydrocarbon liquid. The position of the absorption bands of carotenoids in a monolayer is consistent with this view.

But although the character of the interface as a solvent is sufficient to account for the shift in absorption spectrum of astacene, the evidence at present available is not sufficient to exclude enolization as a contributing factor.

Summary

Astacene in light petroleum is yellow: when the solution is emulsified with water the colour changes to pink. This change is not affected to any marked degree by *p*H or salt concentration. It is suppressed by other surface active substances, with the exception of the anions of fatty acids.

The position of the absorption band of astacene in the yellow-red region of the spectrum has been measured in different solvents. In more polar solvents the band is further towards the red region. The position of this band, in light petroleum solution and in emulsified light petroleum solution, has been measured for astacene, β-carotene and zeaxanthin. In all cases the band is shifted to longer wave-lengths by emulsification, the effect being least with carotene, greatest with astacene.

Surface pressure and surface potential measurements have been made on monolayers of astacene, and of astacene mixed with oleic acid. On acid solution there is little interaction in the monolayers between the two sets of molecules: on alkaline solution there is a pronounced interaction.

The cause of the colour change on emulsification is discussed. It is attributed mainly to the more polar character of the interface compared with light petroleum, but in the case of astacene enolization may also be involved.

We are indebted to Dr Kidd for his kind assistance in obtaining a supply of lobster eggs; to Mr McBey for obtaining eggs under conditions of some difficulty; to Mr J. L. Parkinson for construction of the surface potential apparatus; to Mr G. W. Spicer for preparing polonium electrodes; and to Dr J. H. Schulman for reading the manuscript.

REFERENCES

Adam (1938). The Physics and Chemistry of Surfaces. Oxford.
—— Danielli & Harding (1934). *Proc. roy. Soc.* A, **147**, 491.
—— —— Haslewood & Marrian (1932). *Biochem. J.* **26**, 1233.
—— & Jessop (1926). *Proc. roy. Soc.* A, **112**, 376.
Danielli, Marrian & Haslewood (1933). *Biochem. J.* **27**, 311.
Deutsch (1927). *Ber. dtsch. chem. Ges.* **60**, 1036.
Emerson & Fox (1940). *Proc. roy. Soc.* B, **128**, 275.
Fox & Pantin (1941). *Philos. Trans.* B, **230**, 415.
Karrer & Loewe (1934). *Helv. chim. Acta*, **17**, 745.
—— —— & Hübner (1935). *Helv. chim. Acta*, **18**, 96.
Kuhn & Sorensen (1938). *Ber. dtsch. chem. Ges.* **9**, 1879.
Schulman & Cockbain (1938). *Trans. Faraday Soc.* **34**, 748.
—— —— (1939). *Trans. Faraday Soc.* **35**, 1.
—— & Rideal (1937). *Proc. roy. Soc.* B, **122**, 29.
—— & Stenhagen (1938). *Proc. roy. Soc.* B, **126**, 356.

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CONCERNING THE PIGMENTS OF THE TWO-SPOTTED OCTOPUS AND THE OPALESCENT SQUID[1]

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The majority of fishes and invertebrate species of the sea exhibit integumentary colors due either to melanin pigments or, especially in the invertebrates, to carotenoids. Carotenoids are often also relatively concentrated in such tissues as the liver or digestive gland, and in eggs. Chromoproteins, flavines, purines, and certain more rare pigmentary compounds are discussed by numerous writers (e.g. Verne, 1926).

This paper concerns the pigments encountered in various tissues of two cephalopods of Pacific waters, namely the two-spotted octopus, *Paroctopus bimaculatus,* and the common squid, *Loligo opalescens.* Cephalopods studied by Lönnberg (1935) and the two species investigated by us have revealed a striking general absence of carotenoids from the integument and gonads and only traces in the eyes, but there are considerable quantities of these yellow, orange, or red pigments in the "liver"[2] of *Paroctopus.* Melanins characterize the ink of these mollusks, and are present also in the iris and skin. Octopods show relatively large quantities of melanin in their highly specialized chromatophores, while certain squids exhibit very little in the integument, the pigment there often appearing light brown or reddish instead of grey or deep brown as in the former group. We encountered, in the pericardial glands of the octopus, small amounts of yellow flavines as well as a red water-soluble pigment of unknown identity in the kidneys.

Lonnberg (op. cit.), making preliminary studies of the carotenoids of three cephalopods, reports lutein in the eyes of *Sepiola scandica, Rossia macrosoma,* and *Eledone cirrosa.* He found no carotenoids in the eggs of *Rossia* or *Sepiola,* and only possible traces in the testes of *Eledone.* Extracts of *Rossia* eggs, however, gave a strong color reaction with $SbCl_3$, suggesting the possible presence of vitamin A or a similar com-

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 154.

[2] The term as employed here refers to the large red-brown, grey or greenish gland communicating by a pair of ducts with the alimentary canal at the junction of stomach and intestine. Actually the "liver" and "pancreas" are incorporated together, without a very sharp division, in the single gland.

pound. Carotenoids were not detected in the skin of *Sepiola* or *Rossia,* but were reported in the mantle of *Eledone.* Especially notable was the presence of quantities of a carotenoid resembling lutein in the liver of *Eledone.* The livers of the other two species were not given special study.

Escher-Desrivières, Lederer and Verrier (1938) cite older observations of Kruckenberg on the purple pigments in retinae of certain cephalopods. Studying the retinal pigments of *Sepia officinalis, Octopus vulgaris,* and *Eledone moschata,* these authors found certain chemical and spectroscopic similarities between the cephalopod eye-pigments and those of classical types. They found, however, that the cephalopod retinal purple was stable in light, and they failed to find A vitamins in the material. More recent investigations by Wald (1941) have demonstrated vitamin A and retinene in the retina of the squid *Loligo pealii,* but no traces of these or other carotenoids in other squid tissues. According to Wald, vertebrates and invertebrates alike use vitamins A in the eye. Failure by other investigators to identify these carotenoids in the eyes of squids and other invertebrates is attributed by Wald to the low capacity of such forms for storing these substances. Wald points out that the quantity of vitamin A remains constant in the squid's eye in light and darkness, and that it may not participate directly in the visual processes. He claims that the deep purple pigment of the squid retina, sometimes referred to as visual purple, is really "a photostable, alkali soluble probably melanoid pigment," acting as a light-screen for the retinal cells, but probably playing no role in vision proper.

Materials and Methods

Specimens of *Paroctopus bimaculatus* were taken freshly from the sea and killed by immersing in tap water just preceding analysis. Small specimens (about 6 inches in length) of the squid, *Loligo opalescens,* were bought from wholesale fish-dealers in San Diego. The animals showed no signs of decomposition, having been stored on ice since capture a few days earlier.

Brown or black melanin pigments were detected in the various tissues merely by visual inspection.

Flavines were characterized by their solubility in aqueous solutions, insolubility in organic solvents such as petroleum ether, bleaching of the yellow color by the addition of sodium hydrosulfite, reversible on shaking in air, and light absorption in the violet end of the spectrum near 417 mμ.

Carotenoids were extracted, adsorbed on chromatographic columns,

and given chemical and spectroscopic study in accordance with standard methods described elsewhere. (Fox and Pantin, 1941; Scheer, 1940; Fox and Scheer, 1941.)

Results

A preliminary survey, summarized in Table I, shows the distribution of certain of the pigments among various tissues of the two species, and reveals a few arresting facts. In *Paroctopus* we found no sexual dif-

Table I

Distribution of Pigments in the Tissues of *Paroctopus bimaculatus* and *Loligo opalescens*

Species	Tissue	Weight, grams	Carotenoids	Flavines	Melanins
Paroctopus bimaculatus; two specimens, male (742 gm.) and female (778 gm.).	Salivary glands	♂ 3.8	–	–	?
		♀ 3.9	–	–	?
	Crop, stomach and intestine	♂ 4.2	traces	–	–
		♀ 6.4	traces	–	–
	Gills	♂ 6.4	traces	–	–
		♀ 9.1	traces	–	–
	Liver	♂ 13.8	+++	–	–
		♀ 20.1	+++	–	–
	Ink	1 to 2	+	–	+++
	Eyes	♂ 2.1	traces	–	+
		♀ 2.7	traces	–	+
	Gonads	♂ 13.2	traces	–	–
		♀ 2.3	–	–	–
	Pericardial glands	♂ 2.5	–	+	–
		♀ 2.4	–	+	–
	Kidneys*	♂ 1.3	–	···	–
		♀ 1.2	–	··	–
	Heart	♂ 1.3	–	–	–
		♀ 1.3	–	–	–
	Muscle and skin†	♂640	–	?	skin + muscle –
		♀709	–		
	Eggs‡	33.8	traces‡	?	–
Loligo opalescens 7 ♂, 5 ♀	Gills		–	–	–
	Digestive tract		–	–	–
	Ink and sac	2.8 (from 12 animals)	trace?	–	+++
	Eyes	16.5 (from 12 animals)	(+)	?	+
	Accessory nidimental gland	0.9 (from 12 animals)	traces	–	–
	Rest of body (including gonads)		traces?	·····	traces

* The organs, purple in color, yielded quantities of a water-soluble, blood-red pigment discussed in text.

† Acetone extracts of muscle + skin gradually turned a pink-orange color, as did the ground tissue itself. This was not due to either carotenoids or flavines. The pigment was unchanged by acids or salts, but turned yellow in base, and its acetone solution gradually bleached in air.

‡ Laid by another female. Traces of yellow pigment soluble in petroleum ether or $CHCl_3$, gave pink to mauve color in $CHCl_3$ with added $SbCl_3$.

ferences in pigmentation. Here, as in *Loligo,* neither the mature testis nor the ovary or deposited eggs showed more than possible traces of carotenoids. The same was true in gills, digestive tract (minus liver) and eyes, while in the other tissues these pigments were absent. Carotenoids were yielded in considerable quantities by the relatively large liver, however, and were present in appreciable amounts in the ink stored in its sac, attached to the liver but not in direct communication with it. Flavines

were extracted only from the yellowish tripartite pericardial glands (of excretory function), while melanins characterized the ink, iris and skin.

The kidneys of *Paroctopus* were deep purple-red in color and when triturated yielded a blood-red water-soluble pigment. This pigment is insoluble in acetone or alcohol, being reversibly precipitated from water by these solvents. It contains iron and possesses reducing properties. Study of this pigment is being continued, and a more complete account of it will be published later.

Loligo was found to exhibit but little pigment of any kind in most of its tissues. Neither of the mature sexes yielded any pigments in the gonads. Flavines were not detected with certainty, and carotenoids were present in only very slight quantities in the eyes, while the other tissues yielded none or only suspected traces. While the body integument contained very little melanin this pigment was present in both iris and ink in considerable quantities.

Carotenoids in the Liver and in the Ink of the Octopus

The carotenoids were classified according to (1) their behavior in the partition test, both before and after hydrolytic treatment with alcoholic potassum hydroxide: carotenes and xanthophyll esters remain in the upper, or epiphasic layer of petroleum ether, while free xanthophylls migrate to the lower or hypophasic layer of 90 per cent methanol; following hydrolysis, carotenes remain unchanged, xanthophyll esters from the epiphase are split to give free xanthophylls which behave accordingly, while free carotenoid acids are now removed as potassium salts; (2) carotenes were separable one from the other by adsorption on Tswett chromatographic columns of $Ca(OH)_2$, while xanthophylls were resolved into individual pigments on similar columns of $CaCO_3$; (3) finally, absorption spectra of the separate pigments were determined with a Hartridge Reversion Spectroscope or with a Bausch and Lomb Spectrophotometer. Carbon disulfide was employed as solvent in all of the carotenoid observations.

Liver

Carotenes, represented by from two to five colored zones on the chromatogram of the hydrolyzed epiphase, usually showed a fairly prominent red or orange zone near the top, and a yellow zone nearer the bottom of the column. One of the more prominent upper zones gave absorption maxima agreeing closely with those of β-carotene, i.e., 519.5 mμ and 484.4 mμ (Hartridge) and 510 to 512.5 mμ and 482.5 to 485 mμ (B. and

L. spectrophotometer). Another prominent carotene-like pigment appeared above the β-carotene zone, with absorption maxima at from 505 to 507.5 mμ and 480 mμ. At times the shorter wave-length maximum had higher values of 484 to 486 mμ. We did not identify this pigment with any known carotene.

Xanthophylls, present both free and as esters, were represented by considerable quantities of lutein (505 to 508 μ and 474.5 to 479 mμ by Hartridge; 505 to 510 mμ and 477.5 mμ by spectrophotometer), and by lesser amounts of other pigments of the taraxanthin or eloxanthin range of spectra (500 to 505 mμ and 469 to 473 mμ); occasionally a third type was encountered, below the other two on the chromatogram, with absorption maxima not unlike those of antheraxanthin or petaloxanthin, i.e., 512 mμ and 481 mμ (cf. Strain, 1938).

Acidic carotenoids, appearing only after hydrolytic treatment, occurred in both the epiphase and hypophase.[8] They always presented a single, broad and asymmetrical maximum, difficult to determine with precision in the Hartridge instrument, but giving thereby values from 501 to 507 mμ, and, with the spectrophotometer, a broad hump between the values of 500 to 507 mμ, with a slight maximum at about 503 mμ.

Ink

Carotenes were absent.

Xanthophylls, both free and esterified, were found in smaller quantities than in liver tissue. Hydrolytic treatment did not permit subsequent recovery of sufficient quantities for critical spectroscopic study. Inspection of the fresh ink extract, however, revealed, in one instance, absorption maxima similar to those of lutein (510 mμ and 475 mμ). The epiphasic pigments of the ink carotenoids dissolved in carbon disulphide gave an orange solution of esters with maxima at 502.6 mμ and 472 mμ (cf. eloxanthin), while the hypophasic pigments in carbon disulfide failed to yield sharp maxima. Hydrolysis of the epiphase yielded free xanthophylls accompanied by the familiar carotenoid acid. The hypophase yielded, on hydrolytic treatment, an acidic carotenoid, sometimes but not always accompanied by a non-acidic xanthophyll.

In Table II are presented data concerning the relative mass of liver tissue and the range of concentrations of carotenoids encountered in liver and ink. Since the preponderant carotenoid of both liver and ink exhibited, in chemical, adsorptive, and spectroscopic behavior, properties agreeing closely with those of lutein, the concentrations of mixed carotenoids were measured, for purposes of comparison, in "lutein equivalents" with a Bausch and Lomb visual spectrophotometer.

[8] Always hypophasic after hydrolysis of the extract.

The combined weight of liver and ink is seen to vary between 1.86 per cent and 3.29 per cent of the total body weight, with a mean value of about 2.7 per cent. We were rarely able to recover more than one or two grams of the ink, which represented from 5 to 11 per cent of the combined weight of liver, ink-sac and ink.

The concentration of carotenoids (in lutein equivalents) in liver tissues ranged between 1.2 and 8.3 mg. per 100 gm. with a mean value of about 3.5, in freshly excised tissue from normal animals, i.e., excluding the relatively low value of 0.8 given by the autolyzed liver of animal No. 7. The ink of freshly caught specimens yielded appreciable amounts of

TABLE II

Concentrations of Carotenoids in Liver and Ink

Animal (sex)	Weight, grams	Liver + ink weight, grams	Liver and ink % of total body weight	Total carotenoids (as lutein) mg./100 g.
1 ♂	742	13.8	1.86	2.4
2 ♀	778	20.1	2.58	2.1
3 ♂	716	20.6 (liver)	3.19	1.2
		2.3 (ink)		0.70
4 ♂	721	18.9 (liver)	2.77	3.9
		1.1 (ink)		0.55
5 ♂ (starved)	820	25.4 (liver)	3.29	.....[1]
		1.6 (ink)		0.0
6 ♂ (immature)	493	12.7	2.57	8.3[2]
7 ♂	623	15.1	2.42	0.82[3]

[1] Very little of the pigment in this specimen was carotenoid, having evidently been altered by starvation.

[2] Quantity of carotenoids unchanged by incubating toluene-preserved macerated liver at its natural pH (5.42) for 44 hours at 37° to 40° C.

[3] Liver + ink autolyzed by placing in closed flask in presence of a few drops of $CHCl_3$ for 16 days at room temperature.

carotenoids showing values of from 0.55 to 0.7 mg. per 100 gm. (mean value from two determinations 0.62 mg. per 100 gram) while the ink of one specimen (No. 5) which had been starved for two or three weeks was found to be completely lacking in carotenoids.

DISCUSSION

Regarding the chief role of the liver-pancreas in cephalopods, Jordan (1929) states that it possesses only a secretory function, elaborating digestive enzymes which are passed into the gut during feeding.

In its lack of absorptive function, and its secretion of extracellular enzymes, the liver of cephalopods differs markedly from that of lamellibranch mollusks (see Yonge, 1931). The California mussel, a typical

phytoplankton feeder, passes great amounts of minute plant plastids into its digestive diverticulum, or "liver," whence certain cells engulf and absorb food, passing the undigested residues back into the digestive tract (Fox et al., 1936; Scheer, 1941, and unpublished observations on voiding of feces during early starvation). It would appear obvious that the liver of the mussel receives its rich supply of carotenoid pigments in a direct way from the plant material taken in. The liver of the octopus, on the other hand, probably receives its supply of carotenoids through the blood stream.

Very little seems to be known of the physiology of ink secretion in cephalopods, or of the biochemistry of the ink itself, beyond its rich melanin content. Its carotenoid content provokes much thought. We are aware of the loss of carotenoids from ectoderm in various ways, such as the sloughing of skin, the growth of feathers in certain birds, the discharge of ear-wax in cattle, and the presence of such pigments in the cores of solid materials secreted from the femoral pits of iguanas (unpublished data). Save for the storage of carotenoids in egg-yolk of many oviparous vertebrates and invertebrates, and the secretion of such pigments in the milk fat from mammary glands, the discharge of carotenoids from an internal structure to the outside would appear to be rather unique.

Carotenoids of *Paroctopus* disappeared from both liver and ink in a starved specimen, yet were reduced in amount only very slowly on autolysis of the liver at room temperature, or by incubation of the ground tissue at 37° C.

Clearly, a thorough investigation of the physiological role of the liver is called for, likewise a biochemical study of the ink and its relationship to the metabolism of the animal as a whole, with particular reference to the liver.

Summary

The kinds and tissue-distribution of various pigments in the cephalopods *Paroctopus bimaculatus* and *Loligo opalescens* are described and discussed. Melanins, stored in quantity in eyes and ink of both species, are far more plentiful in the integument of the octopus than in that of the squid.

Flavines were detected with certainty only in the pericardial (excretory) glands of the octopus. This species also yielded, from its kidneys, a water-soluble, iron-containing red pigment with reducing properties.

Carotenoids were present only in traces in any of the organs of the squid. This was likewise true of the octopus, save in the liver-pancreas of this species, which contained a variety of carotenoids in amounts of

3.5 mg. (lutein equivalents) per 100 grams of moist tissue (average value). Ink from the sac adjacent to the liver contained 0.55 to 0.70 mg. lutein equivalents per 100 grams.

In the liver of *Paroctopus,* free and esterified xanthophylls, predominantly of the lutein class, were accompanied by smaller amounts of other xanthophylls, β-carotene and an additional unfamiliar carotene, and a unique carotenoid acid, appearing on hydrolysis, not identical spectroscopically with astacene or with the metridene of Fox and Pantin.

The ink of this species yielded no carotenes, but free and esterified xanthophylls and (on hydrolysis) a carotenoid acid similar to respective compounds recovered from the liver. Whilst starvation brought about the gradual disappearance of carotenoids from liver and ink, autolysis of the liver or incubation of its ground tissues resulted in only slow loss of the pigments.

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LITERATURE CITED

Escher-Desrivieres, J., E. Lederer, and M. L. Verrier, 1938. *Comptes Rendus Acad. Sci.,* **207**: 1447–1450.

Fox, D. L., et al., 1936. The habitat and food of the California sea mussel. *Bull. Scripps Inst. of Oceanography,* **4**: 1–64.

Fox, D. L., and C. F. A. Pantin, 1941. The colours of the plumose anemone, Metridium senile (L). *Phil. Trans. Roy. Roc. London.* B. **230**: 415–450.

Fox, D. L., and B. T. Scheer, 1941. Comparative studies of the pigments of some Pacific Coast echinoderms. *Biol. Bull.,* **80**: 441–455.

Jordan, H. J., 1929. Allgemeine vergleichende Physiologie der Tiere, Walter de Gruyter & Co. Berlin, 761 pp.

Lönnberg, E., 1935. On the occurrence of carotenoid substances in cephalopods. *Arkiv för Zoologi.* 28B, No. **8**: 1–4.

Scheer, B. T., 1940. Some features of the metabolism of the carotenoid pigments in the California sea mussel (Mytilus californianus). *Jour. Biol. Chem.,* **136**: 275–299.

Strain, H. H., 1938. Leaf Xanthophylls. *Carnegie Inst. of Washington Publ.,* No. 490, 147 pp.

Verne, J., 1926. Les Pigments dans l'Organisme Animal, Gaston Doin et Cie Paris, 608 pp.

Wald, George, 1941. Vitamins A in invertebrate eyes. *Amer. Jour. Physiol.,* **133**: 479–480.

Yonge, C. M., 1931. Digestive processes in marine invertebrates and fishes. *Jour. du Cons. Internat. pour l'Expl. de la Mar.,* **6**: 175–212.

Contribution No. 155

Reprinted from SCIENCE, February 20, 1942, Vol. 95, No. 2460, pages 193–194.

MEAN SEA LEVEL AND SAND MOVEMENT; A REPLY[1]

SPECULATIVE hypotheses which overlook factual evidence are luxuries which might well be dispensed with during the war, particularly where conclusions might lead to errors of judgment in military construction. A serious case of this nature appeared recently in this journal[2] and was the less excusable because its writer had been informed of the facts. In this article Leypoldt attempted to show that observations by U. S. Grant and by the writers relative to seasonal offshore shifting of sand in winter and onshore shift in summer have been based on erroneous interpretations, and that actually the sand is shifted along the shores of bays due to changing currents piling up at one end of each bay during one season and at the other end during another season. Leypoldt gives the impression that these shifts of the sand are well known and that the out- and in-movement of sand is nothing but an unwarranted assumption.

These arbitrary statements by Leypoldt will be a surprise to every one who has studied the California beaches either from a scientific or engineering point of view. According to Leypoldt, the sand beaches should be as wide on the average in summer as in winter, but the wide zones should be present at different places. This can be said to be absolutely contrary to fact. The writers have made over 50,000 soundings and observations on the width of beaches at La Jolla and elsewhere along the California coast during the past nine years. Short-interval observations have been made for the past four years along the entire length of the beach south of the Scripps Institution of Oceanography at La Jolla and for over

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 155.

[2] Harry Leypoldt, SCIENCE, 94: 2452, 607–609, 1941.

a year LaFond has measured the depths along the three open ocean piers in the San Diego area at frequent intervals. Grant and his students have made seasonal measurements of beach profiles at a number of places along the coast near Los Angeles. Also the Los Angeles County engineers have measured profiles off the beaches in Santa Monica Bay at different seasons. The results have been remarkably similar. In nearly all cases the sand level has shown a decrease along the shore during winter and an increase outside. Numerous small beaches in coves of rocky headlands are completely washed out during the first winter storms and do not return until there has been a long period of small waves. In some cases the beach is removed and rebuilt several times during a season, provided there are long calm periods alternating between storms.

On the other hand, there is much evidence of temporary shifting of sand along the beaches and there are times when cutting at one part of a beach is accompanied by fill along another portion. The records obtained from the beach extending south of Scripps Institution show cases of this nature and the observation of currents at the end of the pier show the reason for the shift of the sand. However, these lateral shifts are on the whole subordinate to the outward and inward movements of the sand as evident from the shoaling and deepening of water recorded several hundred feet from shore.

Leypoldt appears to be disturbed by the idea that oscillating wave motions are accompanied by shifting of sand both toward the land and toward the sea. The study of rip currents[3] gave some data in this regard. Also the study of the effects of different kinds of waves[4] in relation to the shifting revealed that large

[3] F. P. Shepard, K. O. Emery and E. C. LaFond, *Jour. Geol.*, 49: 337–369, 1941.

[4] E. C. LaFond, *Proc. 6th Pacific Science Congress*, II: 795–799, 1939.

waves of long period shift sand seaward most effectively, whereas small waves of small amplitude shift sand towards the land.

Leypoldt's hypotheses as to the cause of seasonal fluctuation in sea-level heights are as ungrounded as are those concerning sand movements. He states, "Sea-level heights are functions of rainfall in the locality, together with river discharge and other methods of ground-water return, and water removal for rainfall." Observations from the tide staff at La Jolla show that sea level here is usually highest during the months of least rainfall, when the Southern California rivers are dry, and when the water has its highest salinity. The more plausible explanation of sea-level fluctuation is based upon the fluctuations in water temperatures.[5]

Other statements by Leypoldt, such as "When sea level is high along the Pacific coast of the United States it is low along the South American coast, necessitating an interchange of water between the hemispheres," are made without giving any data whatsoever, so that one wonders if they are not also based on some of Leypoldt's hypothetical hunches. It is to be hoped that in the future Mr. Leypoldt will make some attempt to investigate the facts before he lets these wild ideas loose on the scientific public.

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[5] E. C. LaFond, *Jour. Marine Research*, 2: 1, 17-29, 1939.

Contribution No. 156

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Vol. 70, No. 3, May, 1942

THE REPRODUCTIVE ORGANS OF THE PROSOBRANCH MOLLUSK CREPIDULA ONYX AND THEIR TRANSFORMATION DURING THE CHANGE FROM MALE TO FEMALE PHASE[1]

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FIVE FIGURES

In previous papers the writer (Coe, '35, '36, '38 a, '38 b) and others (Kleinsteuber, '13; Giese, '15; Gould, '17; Ishiki, '36, '39; Moritz, '38) have described the gonads and associated sexual organs in several species of this and related genera, including the transformations which occur during the change of sexual phase. Yet the morphological and physiological changes which occur during the transformation of sex in these mollusks are so profound and so unique that it may be of interest to subject them to more thorough examination.

Cerpidula onyx Sowerby and its deviating habitat-form C. rugosa Nuttall are abundant along the coast of Southern California. The former is usually found lying on muddy bottoms in pyramidal groups of three to sixteen superimposed individuals, while the latter occurs singly or in groups of two to four attached to large mollusk shells and rocks (Coe, '38 b).

In this species as in several others of the genus, the eggs are laid in gelatinous capsules and incubated for a period of about 2 to 3 weeks beneath the body of the parent. The

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 156.

larvae are then set free in the water and feed on plankton for about 2 weeks more before settling down and transforming to the adult condition. Each of the 50 to 100 capsules which are deposited at each ovulation contains 100 to 200 ova, making a total of 5000 to 20,000. As many as six to ten ovulations may occur in 1 year on the coast of Southern California. Only part of the ova reach even the free-swimming stage, however; frequently fully half the entire number of embryos disintegrate within the capsules and are used as food by the survivors.

DEVELOPMENT OF PRIMARY GONAD

The primary gonad, which begins development immediately after metamorphosis, is distinctly ambisexual in nature. But since the spermatogonia proliferate much more rapidly than the ovogonia, the gonad soon assumes the general appearance of a spermary. A few larger vesicular nuclei indicative of ovogonia and ovocytes are always present among the basal cells of the gonad. Some of these remain as the antecedents of the germinal cells of the future ovary while others degenerate and disappear during the process of spermatogenesis.

MALE PHASE

This species is typically protandric, all normal individuals functioning as motile males for 2 months or more or until the body has reached a length of about 10 mm. If suitably mated the male phase may continue much longer, occasionally until the body exceeds 30 mm. in length. The female phase which follows is functional throughout the remainder of the animal's life, sometimes of 2 years or more. The body may then exceed 60 mm. in length (Coe, '38 b).

Spermatogenesis proceeds so rapidly that apparently ripe spermatozoa may be present in the gonad when the animal is only a few weeks of age and only 4 to 5 mm. in length. At 6 mm. the seminal vesicles may be well filled with sperm. At this stage the slender copulatory organ (penis) begins to grow out from the body wall behind the right tentacle (fig. 1).

The growth of the penis always follows, rather than precedes, the onset of spermatogenesis. The individual thereby assumes the characteristics of the male phase not only in structure but in behavior. It should be mentioned, however, that Moritz ('39) found that the penis is present at the time of hatching in C. adunca, although the gonad could not be detected.

The young male if isolated moves about restlessly, particularly by night. If another male is present the young male

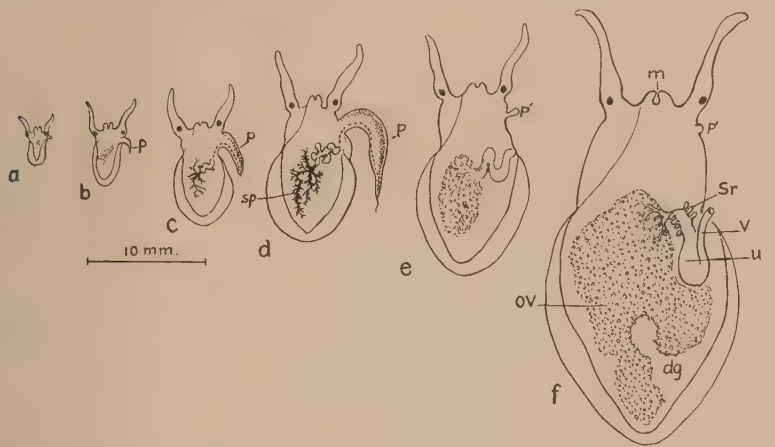

Fig. 1 C. onyx. Development of reproductive organs in male phase (a, b, c, d), transition stage (e) and female phase (f), all drawn at same scale; letters indicate: dg, digestive gland; m, mouth; ov, ovary; p, penis; p', rudiment of former penis; sr, seminal receptacles; u, uterus; v, vagina.

attaches itself upon the other male's shell, but only temporarily if a female is accessible. If an individual in the female phase is encountered the attachment may continue during the entire male phase or even after the change of sex and then necessarily throughout life.

Spermary. The male gonad consists of a series of branching tubules which have grown out from the primary gonad and which have later spread out along the left side of the visceral mass (figs. 1, 2).

The constituent spermatogonia multiply rapidly and form both eupyrene spermatozoa and apyrene spermatosomes, as

previously described for other species (Gould, '17; Coe, '36). These cells soon fill the entire lumen of the tubule (fig. 3, A), whence the mature sperm cells of both types pass into the seminal vesicles.

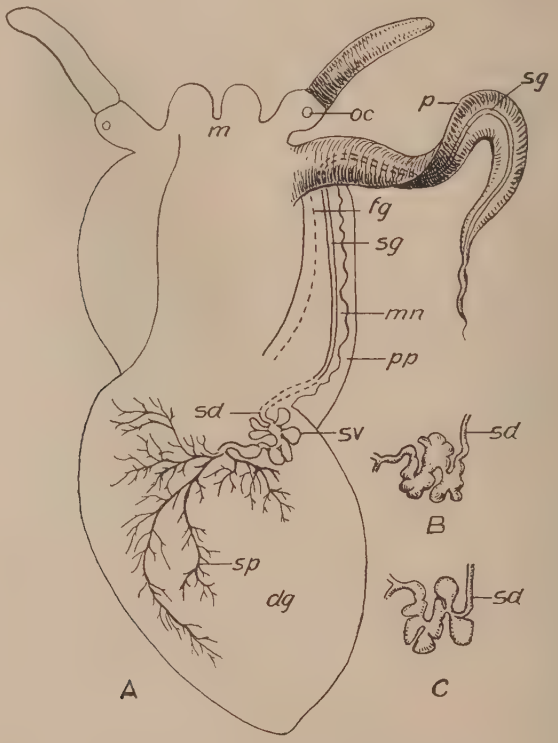

Fig. 2 C. onyx. A, Reproductive organs of functional male, showing spermary (sp), sperm duct (sd) and seminal vesicles (sv), with sperm groove (sg) near border of mantle (mn) and extending along ventral surface of penis (p). Other letters indicate: dg, digestive gland; fg, food groove; m, mouth; oc, ocellus; pp, propodium. B, C, Less sacculated seminal vesicles of other individuals.

Sperm duct and seminal vesicles. The branches of the spermary unite proximally to form the single sperm duct. The latter gradually becomes enlarged, convoluted and sacculated, thereby forming the seminal vesicles (figs. 1, 2). Here the spermatozoa accumulate until an opportunity for copula-

tion is afforded. They then pass outward through a short proximal portion of the sperm duct and then enter the sperm groove on the right dorsal side of the neck lappet.

The sperm groove is a narrow, ciliated trough which continues forward to the base of the penis and thence on the ventral side of the penis to its tip. At the time of copulation the tissues around the sperm groove become swollen and the groove deepened so as to form an almost closed tube. When fully functional the penis is about two-thirds as long as the body and its tip becomes extended into a delicate sensory filament (fig. 2).

The extent of spermatogenesis and the length of the male phase depend both upon the hereditary composition of the individual and the environmental conditions. Some individuals are of the feminine type and have but a relatively brief male phase even under the most favorable conditions. Others, of a more masculine type, retain this phase much longer under similar circumstances. Opportunity for mating usually results in an accentuated and prolonged male phase but association with another individual is not necessary for the development of that phase (Coe, '38 a, '38 b). Unfavorable environmental conditions as well as isolation usually terminate the male phase within a few weeks.

TRANSITION PHASE

Toward the termination of the male phase spermatogenesis gradually ceases. Most of the spermatozoa of both types then pass to the seminal vesicles. Those that remain, as well as the immature spermatogenic cells soon begin to undergo disintegration and cytolysis (fig. 3, B). Only the residual ovogonia and a few ococytes remain on the walls of the collapsed gonadal tubules (fig. 4, A, B).

The seminal vesicles remain distended with spermatozoa and apyrene spermatosomes and copulation may take place for a brief period after the cessation of spermatogenesis. Soon, however, the apyrene spermatosomes in the vesicles

undergo disintegration, followed by phagocytosis of the functional sperm by the epithelial cells lining the vesicles. At this time the penis becomes smaller and is eventually resorbed into body, except for a small, more or less persistent knob (figs. 1, 5).

Fig. 3 C. fornicata. A, Portion of gonad of functional male, showing development of eupyrene (spz) and apyrene (aspz) spermatozoa, with two ovocytes (oc) in basal syncytium (bs); other letters indicate: ug, undifferentiated gonia; spg, spermatogonia; spc^1, spc^2, primary and secondary spermatocytes; spt, spermatids. B, Gonad in early transition stage, with only a few spermatozoa remaining; d, disintegrating spermatic tissues. (From Coe, '36.)

There is thus a sharper histological demarcation between the male and female phases of the gonad than has been reported for most of the other species of the genus. During the transition stages the individual often returns to a state of sexual immaturity, for the functional male phase may have passed before the female phase begins. This is also the period of the animal's most rapid increase in size.

FEMALE PHASE

A period of 3 to 4 weeks usually intervenes between the functional male and female phases.

Ovary. Following the phagocytosis of all the spermatic cells, the tubules of the former spermary increase rapidly in length and form such extensive branches that they cover almost the

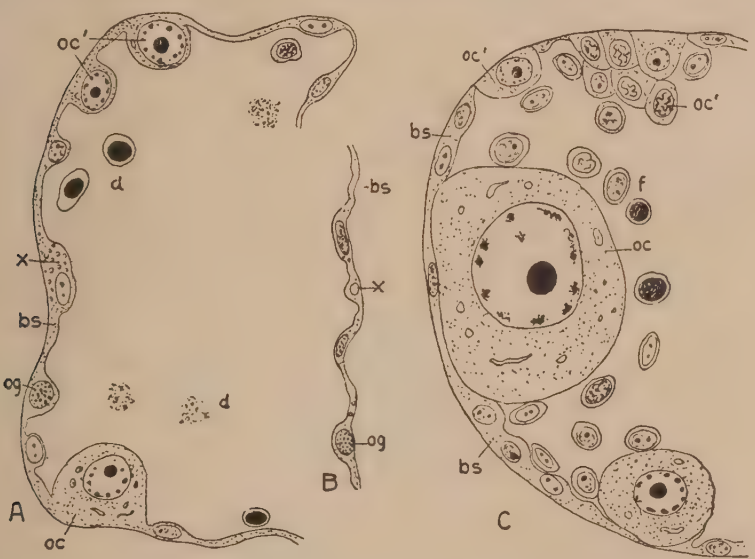

Fig. 4 C. fornicata. A, Portion of gonad of individual in late transition stage, showing thin follicular wall with scattered ovogonia (og) and ovocytes (oc) and the remains of disintegrated cells (x); no spermatozoa remain but the lumen contains a few disintegrating spermatic cells (d). B, Portion of wall of follicle devoid of ovocytes. C, Portion of gonad of individual in early female phase, showing one large and one small ovocyte remaining from previous male phase and smaller ovocytes (oc) in early growth stages, with follicle cells (f) scattered in lumen. Other letters as in figure 3. (From Coe, '36.)

entire visceral mass (figs. 1, 5). The tubules, which at first are slender, are lined with ovogonia and a few rapidly growing ovocytes surrounded by follicle cells (fig. 4, A). As the ova increase in size the tubules become greatly distended and sacculated. They then bear little resemblance to the slender spermatic tubules from which they were derived (fig. 4, C).

Genital duct. During the transition stages the formerly male genital duct becomes differentiated into oviduct, uterus, seminal receptacles and vagina. Perhaps the gonopericardial duct should be included, although this has a separate origin as explained below.

The convolutions and saccular enlargements of the seminal vesicles are straightened out to form the oviduct, while the terminal portion of the sperm duct become greatly enlarged. This, with the adjacent part of the spermatic groove, becomes differentiated into uterus and vagina. A thin-walled fertilization chamber is then formed at the junction of oviduct and uterus and from this chamber a compact group of twelve slender outgrowths form the seminal receptacles. Each of these remains connected with the fertilization chamber by a separate slender canal (fig. 5).

The seminal receptacles are so small that they can be distinguished only by microscopic sections when empty but each of them becomes a conspicuous white, egg-shaped vesicle when filled with sperm. Usually only part of the receptacles receive sperm at the first mating and one or more of the twelve may remain empty in individuals that have been mated for several months.

At the time of copulation both apyrene spermatosomes and true spermatozoa are transferred to the uterus but only the latter enter the seminal receptacles. The spermatosomes disintegrate rapidly in the uterine cavity. The uterus is provided with thick, deeply folded walls consisting of an outer layer of connective tissue and muscular fibers and an inner layer of highly columnar ciliated and glandular epithelial cells. The secretions of these epithelial cells produce the tough and transparent egg capsules on contact with the sea water.

The vagina has thin, distensible walls and is so highly muscular that it can be extended to the edge of the shell in copulation. It also acts as an ovipositor in fastening the egg capsules upon the object to which the animal's foot is attached.

Gonopericardial duct. At an early stage in the sexual transformation a strand of mesenchyme cells grows out from the forming oviduct and joins the wall of the pericardium. This

strand is later differentiated into a ciliated tubule, the gonopericardial duct (fig. 5), as described by Giese ('15), and Gould ('17) for other species. The oviduct is thereby brought into direct communication with the cavity of the pericardium.

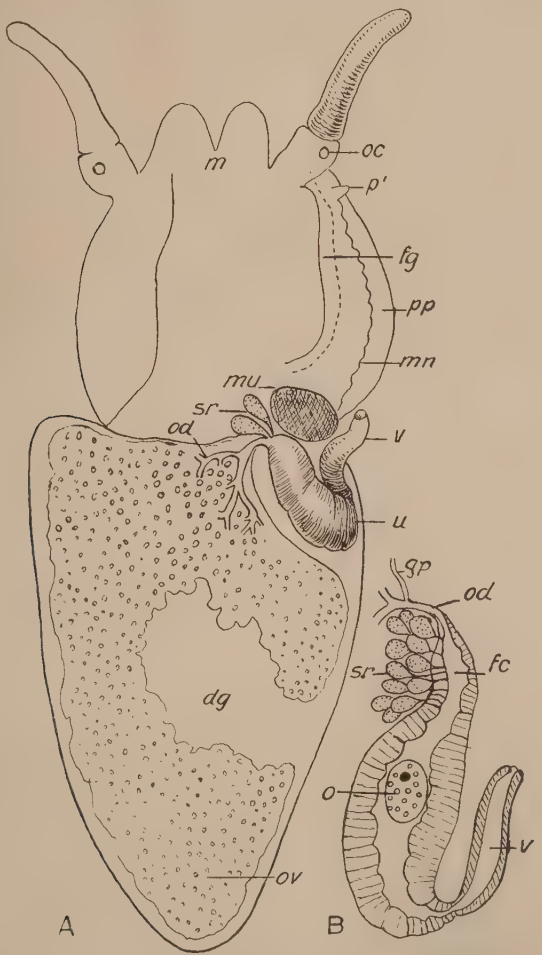

Fig. 5 C. onyx. A, Reproductive organs of functional female, showing uterus (u), vagina (v), two of the twelve seminal receptacles (sr), oviduct (od), gonopericardial duct (gp), ovary (ov), vestige of the former penis (p'), and right shell muscle (mu). Other letters as in figure 1. B, Diagrammatic sections of uterus and vagina showing the twelve seminal receptacles (sr), each opening by a slender tubule into the fertilization chamber (fc); the uterus contains a single ovum (o).

Hence it is presumable that some of the fluid from the latter may mingle with the secretions of the oviduct and uterus at the time of ovulation

LONGEVITY OF SPERM

Several males commonly participate in filling the seminal receptacles of each female. Supplementary males, if present nearby, move to the mating position temporarily even when the female has a semipermanent mate.

The sperm were found to remain functional for more than a year and in sufficient numbers to fertilize all the 50,000 to 200,000 eggs produced in the meantime. This was proved by isolating previously mated females in separate glass containers in a suspended box immersed in the sea and examining them at frequent intervals. Each female attached herself to the inner surface of the glass, whereby the successive clusters of developing eggs could be observed without disturbing the parent.

At the end of 360 days the seminal receptacles were still well filled. How much longer the sperm would have remained functional could not be determined because of the accidental termination of the experiment by a severe storm. It seems quite probable, as Conklin has suggested for another species, that they would have survived as long as the female lived. A survival of 2 to 3 years has been reported for the sperm of certain other gastropods.

DISCUSSION

It is now appropriate to inquire as to the significance of the profound and unique morphological and physiological transformations that have been described on the preceding pages. There are few, if any, similar examples among animals where an organ system which is so highly differentiated for a particular function becomes later dedifferentiated and finally redifferentiated to serve a different function. It should be noted that the sexual conditions in this species of Crepidula do not represent hermaphroditism in a strict sense but rather protandric consecutive sexuality. And this is of such a nature that the individual reaches sexual maturity in the male phase

very early in life. The appropriate organ systems become functional, with associated behavioristic patterns characteristic of the male, when the individual is only 4 to 6 mm. long and only a few weeks of age.

The length of the male phase depends both upon the hereditary composition of the individual (whether of predominantly masculine or feminine tendencies) and upon the environmental conditions. It can be prolonged by association with another individual in the female phase or terminated by isolation, as well as by a great variety of chemical and physical alterations of the water (Coe, '38 a, '38 b).

At the termination of the male phase the copulatory organ is resorbed and the gonads and efferent ducts, which had become so highly differentiated now return to a simple, dedifferentiated condition of sexual immaturity. Under adverse conditions there may be a resting period of several weeks or even several months before any notable progress toward a new and different type of sexuality is made. The female phase will not be realized until favorable environmental conditions are restored.

Under normal circumstances, however, some parts of the female system are in process of formation before the progressive disintegration of the male structures has been completed. In some other species, notably C. plana (Gould, '17) and C. aculeata (Isiki, '39), as well as occasionally in C. onyx, the two sexual phases may overlap to a considerable extent.

Having completed the male phase even in an aborted manner there is no resumption of that phase again. In this respect the sexual transformation is similar to metamorphosis from the larval to the adult condition in other animals.

In the development of the male phase the gonad is activated first and the accessory sex organs are formed later. The degeneration of the accessory organs also follows the initial stages of disintegration of the spermatic cells. The development of the accessory organs in the early female phase likewise follows, rather than precedes, the proliferation of ovogonia in the gonad. In exceptional cases the degeneration of the penis may be delayed until after the accessory female

organs, including the gonopericardial duct, are almost completely developed.

From the above considerations it would appear that the genetic factors which govern the complex sexual conditions in these mollusks act first upon the gonad and that this in turn may participate in regulating the differentiation, dedifferentiation and redifferentiation of the accessory organs and the associated behavioristic responses. That these morphological and physiological changes are all mediated by internal secretions may be assumed. But there is no satisfactory evidence that any masculinizing hormone or other chemical substance passes from the female to the male or from a larger to a smaller individual, as Gould ('19) and Ishiki ('36) have supposed. Young individuals reared in isolation develop the same masculine characteristics as those which are associated with either young or older individuals.

LITERATURE CITED

Coe, W. R. 1935 Sexual phases in Prosobranch mollusks of the genus Crepidula. Science, vol. 81, pp. 570–572.

———— 1936 Sexual phases in Crepidula. J. Exp. Zool., vol. 72, pp. 455–477.

———— 1938 a Conditions influencing change of sex in mollusks of the genus Crepidula. J. Exp. Zool., vol. 77, pp. 401–424.

———— 1938 b Influence of association on the sexual phases of gastropods having protandric consecutive sexuality. Biol. Bull., vol. 75, pp. 274–285.

Giese, Martin 1915 Der Genitalapparat von Calyptraea sinensis Lin., Crepidula unguiformis Lam. und Capulus hungaricus Lam. Zeitschr. f. wiss. Zoöl., vol. 114, pp. 169–231.

Gould, Harley N. 1917 Studies on sex in the hermaphrodite mollusc Crepidula plana. I. History of the sexual cycle. J. Exp. Zool., vol. 23, pp. 1–69.

———— 1919 III. Transference of the male producing stimulus through seawater. J. Exp. Zool., vol. 29, pp. 113–120.

Ishiki, (Isiki) Hitoshi 1936 Sex-changes in Japanese slipper limpets, Crepidula aculeata and Crepidula walshi. J. Sci. Hiroshima Univ., Ser. B, Div. 1, vol. 4, pp. 91–99.

———— 1939 Histological studies on the sexual organs during sex-changes of Crepidula aculeata and C. walshi. J. Sci. Hiroshima Univ., Ser. B, Div. 1, vol. 6, pp. 103–113.

Kleinsteuber, Hans 1913 Die Anatomie von Trochita, Calyptraea und Janacus. Zool. Jahrb. Suppl., vol. 13 (Fauna Chilensis 4), pp. 385–476.

Moritz, C. E. 1938 The anatomy of the gasteropod Crepidula adunca Sowerby. Univ. Calif. Publ. Zool., vol. 43, pp. 83–91.

———— 1939 Organogenesis in the gastropod Crepidula adunca Sowerby. Univ. Calif. Publ. Zool., vol. 43, pp. 217–248.

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STUDIES OF THE RESPIRATORY METABOLISM OF WARM AND COOL SPRING FISHES [1]

F. B. SUMNER AND URLESS N. LANHAM

I. Introduction

It is generally agreed that the respiratory metabolism (oxygen consumption) of poikilothermal animals tends to vary directly with the temperature to which they are subjected. Less familiar is the existence of a process of thermal adaptation, following transfer from one temperature to another. The immediate effects of such transfer may be succeeded by a "rebound" from the extreme condition first induced back toward the previous condition of the animal. A further illustration of the same principle is the fact that the metabolic rate of fishes at any given temperature may be considerably influenced by their previous temperature experience. Fishes which had been acclimatized to 30°, for example, showed a lower rate of oxygen consumption, when transferred to 20°, than ones which had been kept continuously at 20°, and conversely, transfer from 10° to 20° resulted in a higher rate than that shown by fishes accustomed to the latter temperature. Here, too, any such departure from the normal oxygen consumption for a given temperature is followed, within a few days, by a process of adaptation which tends to equalize the metabolic rate, regardless of previous history (Wells, 1935*b*; Sumner and Wells, 1935; Sumner and Doudoroff, 1938).

An obvious question is whether the metabolic rate of fishes and other poikilothermal animals in nature varies as widely with the temperature of their medium as might be inferred from these laboratory results. Or, may not more far-reaching processes of adaptive modification occur in nature, such that warm-water and cold-water derivatives of the same stock come to have nearly the same rates of metabolism?

Fox and Wingfield [2] compared certain closely related species, or local representatives of the same species, living at different latitudes, and reported varying degrees of thermal adaptation of this sort. In some cases, there was no such adaptation, the two forms under comparison differing widely under their own normal conditions of life, but approximating one

[1] Contributions from the Scripps Institution of Oceanography of the University of California, La Jolla, California, New Series No. 157. Services were rendered in the course of these studies by persons working under W.P.A. Project 65–1–07–2317.

[2] Fox, 1936, 1939*a*, 1939*b*; Fox and Wingfield, 1937; Wingfield, 1939.

another closely when their rates of physiological activity were compared at equal temperatures. In other cases, the rates of these functions were nearly identical in their respective habitats, despite considerable differences of temperature. Here extensive adaptation appears to have taken place.

In 1939 Sumner and Sargent made field studies of the respiratory metabolism of fishes in springs of varying temperature in Nevada. Experiments were conducted upon warm and cool spring representatives of species belonging to two genera, *Cyprinodon* and *Crenichthys* (Sumner and Sargent, 1940). In these studies, the rapidity of death in thousandth-normal KCN was used as an index of the rate of respiratory metabolism, the two, as is well known, being directly correlated.

In both of these genera, the warm-spring fishes, at their accustomed temperature, succumbed far more rapidly than the cool-spring fishes at their accustomed temperature. As regards the behavior of warm-spring individuals, transferred to the cool spring, the two fishes seemed to give different results, though the data hardly warrant definite conclusions on this point. It should be pointed out, however, that the seeming lack of adaptive modification of the metabolic rate on the part of *Crenichthys* in warm water has been borne out by the studies to be reported herewith. Its striking resistance to the lethal effects of high temperature is something quite distinct (Sumner and Doudoroff, 1938).

The importance of the physiological and ecological problems here involved, and the widespread human threat to the continued availability of warm springs to naturalists (Brues, 1928) seemed to warrant a second trip to the springs previously visited in our studies of *Crenichthys*. This work was carried on during portions of September and October, 1941.

While we believe that the publication of our present results calls for no apologies, it should be pointed out that field studies of this sort should not be judged by the same standards as are applied to similar studies in the laboratory. This is because of the impossibility of transporting important articles of laboratory equipment, unfavorable weather conditions, and limited time (in this case, less than 4 weeks) at the scene of our operations.

We must here acknowledge valuable help received at various times from our colleagues, Dr. Denis L. Fox and Dr. Marston C. Sargent, and from our companion in camp, Mrs. Margaret Sumner.

II. Methods

Altogether, 200 fishes were employed in these studies, while more than 200 oxygen determinations were made. The fishes were readily caught

at both of these springs with hand-nets. Some time (several hours to a day or more) before the respiration tests were made, small lots (6 to 9) of the fishes, of roughly uniform size, were placed in each of four glass tubes having a length of 40 cm. and an internal diameter of 4 cm. Except during the period of a test, wide-mesh cloth screen was fastened over the flanged ends of the tubes, which were laid, side by side, in a screen cage, placed at the bottom of the stream, and directly in the line of the current. When a test was to be made the screens were removed from one of the tubes, and close-fitting rubber stoppers, one of these carrying a glass stopcock, were forced into its ends. The tube was then returned to its former position in the cage. The capacity of these tubes, with the stoppers in place, ranged from 439 to 454 ml.

At the close of 15 minutes (10 in some experiments) the water-samples were drawn off into glass-stoppered bottles of about 150 ml. capacity. A glass tube, continuous with the stopcock, was inserted into the bottle, down to its bottom, and the water allowed to overflow from the bottle's mouth. Two samples were invariably drawn in each test.[3] Time records, in seconds, were kept of each of these steps with a stop-watch. Within a few minutes of the drawing of each sample, the Winkler reagents were introduced into the bottles. Titration was performed on either the same or the next day.[4]

In recent studies of oxygen consumption in fishes (cf. Keys, 1931; Wells, 1935*a*; Wunder, 1936; Leiner, 1938) it has been the more usual practice to hold the animals in a continuous current of water, and to draw samples from this at intervals, rather than to hold them, tightly stoppered, in an inclosed volume of water. Under the former conditions, the accumulation of carbon dioxide and other possible metabolites is, of course, prevented. Likewise the fishes are subjected to less disturbance in the taking of water samples. It is obvious, however, that such procedure would be difficult, if not impossible, to employ in the field. Moreover, the highest of our CO_2 concentrations probably fell well short of two thousandths of an atmosphere. No signs of discomfort were visible at the close of the 15-minute period, at least in the cooler water. So far as disturbance is concerned, the fishes showed little evidence of excitement except at the beginning and the end of the test. Most of the time they swam quietly or remained at rest. In this connection, it is worth pointing

[3] The values for oxygen consumption employed in our tables and graphs have been based entirely upon the first of these samples. The second figures have been regarded as less representative for a number of reasons.

[4] No appreciable effect of delaying the titration over night is probable. Readings of four divided samples, titrated on the same and on the following day, gave mean values of 2.48 and 2.52 respectively. These were as close as the average divided samples titrated on the same day.

out that our figures show no more variability for fishes of a given size than do those of Wells (1935*a*, pp. 209–210) in experiments with *Fundulus*, where a running-water system was employed. Indeed Wells has recorded fully as great differences as ours when repeating tests of *the same lots* of fishes.

Before and after the performance of a series of these tests, and frequently alternating with them, water samples were withdrawn from the spring, as near as possible to where the tubes had lain.

Owing to the possible presence of nitrites in our samples (cf. Allee and Oesting, 1934) the modified Winkler method (A.P.H.A., 1936) [5] was used in all cases, with a few exceptions which were performed for the sake of comparison.[6]

Under field conditions, it is hardly to be expected that such high precision will be attained as is possible in the laboratory. Thirty cases in which duplicate water samples were drawn from a single tube revealed a mean difference of 2.2 per cent between them. Lack of homogeneity ("streakiness"), in the water, to be discussed presently, may have had its effect, even within our half-liter tubes.

At the close of each test of oxygen consumption, the fishes involved were dried upon paper towels and weighed. The aggregate weight of each lot, together with the estimated original oxygen titre of the water, the oxygen titre at the close of the test, the volume of water in the tube (allowing for volume occupied by the fishes), and the exact duration of the test, are the data upon which the metabolic rates (ml./gm./hr.) are based.

Unfortunately, the equipment at our disposal did not permit of our collecting a sample of the water admitted to the tube at the moment of commencing each test of oxygen consumption. For this reason, the previous oxygen titre of the water which was actually "breathed" by the fishes in any given experiment cannot be stated with precision. As will appear later, water samples from these springs, taken at different times or in slightly different positions, might differ considerably in their oxygen titre. While this circumstance introduces a considerable margin of possible error into the result of any single titration, we believe that the validity of our chief comparisons is little affected. This (1) because the

[5] Our procedure differed from the procedure therein stated in that in the succeeding steps of the test (Winkler proper) HCl was used instead of H_2SO_4, while manganous chloride was used in place of the sulphate. This is in accordance with the procedure long employed at the Scripps Institution in analysis of sea-water.

[6] It is doubtful whether the modified procedure was necessary, in the case of the water-samples taken directly from the springs. Four divided samples, of which half of each was subjected to the modified and half to the unmodified Winkler procedure, gave mean values of 2.54 and 2.55 respectively.

differences involved in comparing two series of differing history are great in proportion to errors due to uncertain original oxygen content of the water; and (2) because results based upon two different procedures which we have employed in selecting a figure to represent this oxygen content show substantially the same relations.

The most obvious of these procedures (1) was to select for each computation the oxygen value of that water sample which was taken most nearly at the same time as the performance of the metabolism test under consideration. However, there were instances in which no water sample was taken within an hour or more of this test.

The alternative procedure (2) was to employ, as our basis of calculation, the mean titre of all the water samples taken in the position in which the test in question was made.[7] It is obvious that many single values for oxygen consumption obtained in this way would be no more than rough approximations. It is equally plain, however, that the *average* of a series of values thus obtained could not depart far from an average such as we should obtain if the actual oxygen titres of the corresponding water samples were available.

It is gratifying that graphs based upon these two procedures agree in most essential features, though differing considerably in detail (Figs. 2–4). In the text, the mean of the two resulting values will be given.

A further difficulty in postulating any initial oxygen content for the water is the fact that a quite appreciable amount of oxygen was taken out of their medium by the fishes before the commencement of the test, despite the continuous flow of water through the tubes. This current was undoubtedly retarded somewhere by the screens, which it was necessary to fasten over the ends. Whatever the exact extent of this preliminary depletion of the oxygen, we may probably fairly assume that it did not differ very widely from one experiment to another.

III. The Springs

"Preston Spring," the largest of several springs in the vicinity of Preston, Nevada, is really a group of small springs, discharging near together and giving rise to a creek of considerable size. The water in and near the orifices appears to maintain a constant temperature very close to 21° C.

The high variability of the oxygen values for all of the water samples taken at Preston Spring is indicated by Fig. 1. Reference has already been made to the "streakiness" of this water in respect to oxygen content.

[7] Excluding those taken on cloudy days or near sunset, which regularly gave very low values.

While the concentration at the sources appears to be fairly constant (cf. Sumner and Sargent, 1940), it is considerably lower than that in the rest of the stream, even in their immediate neighborhood. Surface aeration and photosynthesis are, of course, responsible for the differences. The mean of two samples, drawn from what appeared to be the largest orifice was 1.96 ml. per litre, while samples taken a few yards away averaged about 2.60 ml. The resulting variegation in the oxygen concentration of these waters was shown by determinations of samples taken in various positions.

It would be quite erroneous, however, to infer that the water with which we filled our tubes for the tests of respiratory metabolism was

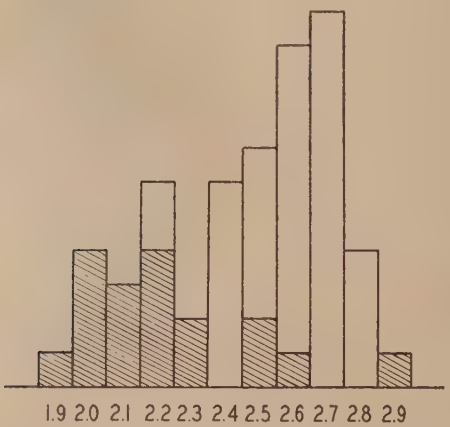

FIG. 1. Oxygen values (ml./l.) for water of Preston Spring. Shaded squares denote samples taken in the first position of our cage directly over one of the minor orifices of the spring; the others in a second position some distance from this.

subject to such an extreme range of variation as is here indicated. Care was taken, moreover, to collect our samples from points close to those where the tests were made.

Some thirty miles to the south, in the same valley,[8] is the warm spring here discussed, known locally as "Mormon Spring." This is a spring of much smaller volume than that at Preston. Its temperature is almost exactly 37° at its source, but the water cools off by a degree or two within a hundred feet downstream. Most of the fishes were caught in water ranging from 35° to 36.5° C., and temperatures such as these prevailed where the experiments were performed. At the lowest point in the stream where fishes were seen, the thermometer recorded 34.8°. Within

[8] Called the "White River Valley," though there is no continuous stream here.

a few hundred feet from its source, the stream spreads out into a marsh, where the water probably follows the temperature of the air. No fishes were found there. It seems probable, therefore, that the Mormon Spring representatives of *Crenichthys baileyi* seldom experience temperatures lower than 34°. On the other hand, individuals were rather frequently seen close to the main source, where the temperature was nearly or quite 37°.

As regards oxygen content, water samples taken well below the surface, in the main orifice of the spring, gave a mean value of 0.51 ml./l., while at a nearby point, where fishes were often seen, this value was 0.65. Samples taken downstream in the creek, where most of the fishes were caught, ranged from 1.54 to 1.88.[9]

IV. The Fishes

Crenichthys baileyi (Gilbert) [10] is the commonest species of fish in Preston Spring, at least near the sources. Besides *Crenichthys*, there are, however, at least three other fishes present. Two of these (*Apocope osculus* and *Lepidomeda sp.*) are Cyprinidae, the other (*Pantosteus sp.*) being a Catostomid. At Mormon Spring, *Crenichthys* is the only fish present. Likewise, it is much less abundant than in Preston Spring. Dr. Hubbs informs us that he regards the two populations of *Crenichthys* here considered as subspecifically distinct. He states (cf. Hubbs and Kuhne, 1937, referring to similar differences in *Apocope*) that the warm spring form has a slightly lower average number of fin-rays than the cool spring form, and counts which we have made of the dorsal and anal rays of about fifty fishes of each race confirm this statement.

Identification of the sexes is difficult or impossible in the smaller specimens mostly used in these studies. Since such individuals were probably sexually immature, it is not likely that sexual differences occurred in their metabolic rate.

V. Oxygen Consumption

Experiments were performed in which each stock was tested in its own waters, and in which each was tested after transfer to the habitat of the other.

Preston at Preston

When all of these fishes are thrown together as a single population (Fig. 2 and Table I), the values for respiratory metabolism (ml./gm.-

[9] Readings for these were as follows: 1.54, 1.56, 1.62, 1.76, 1.78, 1.86, 1.86, 1.88.

[10] Cf. Hubbs, 1932; Hubbs and Miller, 1941. We are indebted to Dr. Hubbs for the identification of the other species.

/hr.) are seen to vary very widely, ranging from 0.123 to 0.390, using in each case the mean of the values obtained by our two procedures (see p. 317). This variation is in part due to the heterogeneous nature of the material. The series includes fishes differing widely in size, this ranging from 0.51 gm. (mean of one lot of 9 individuals) to 9.5 gms. (weight of one large individual tested alone). While the (inverse) correlation between size and metabolic rate is not very close, Figs. 2 and 3 show that

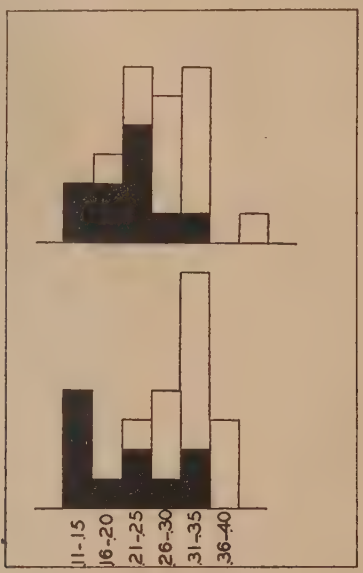

FIG. 2. Rate of oxygen consumption (ml./gm./hr.) of Preston Spring fishes in Preston Spring. Upper histogram is based upon computations (1) in which the O_2 value of the water sample taken at most nearly the same time as the test was used in each case; lower histogram upon computations (2) in which the mean of the entire series of O_2 values (with certain exceptions) was used (see p. 317). Shaded squares denote rate for fishes larger than 1 gram in mean weight, others for fishes less than 1 gram.

such correlation exists. It will be found, too, that the mean of ten values for fishes averaging over 1 gm. in weight is 0.215, that of the thirteen values for fishes below this mean weight being 0.307 (see Fig. 2).

In two cases, special experiments were made with Preston-at-Preston lots, in order that more strict comparisons with the Mormon-at-Mormon series might be possible. Thus, in the case of four lots (designated by asterisks) the period of the test was abbreviated to ten minutes (see be-

low) instead of fifteen minutes as was the rule in the Preston Spring fishes. Again, four other lots (designated by daggers) were tested only a few hours (1½ to 4) after being confined in the tubes, instead of being previously kept through one night, as was commonly done. While the results of both of these tests indicated considerable differences from the general average (13 to 18 per cent), part of this difference appears to

TABLE I

Oxygen Consumption of Fishes (First Sample Drawn)—Preston Spring

(Arranged in order of mean weight)

Lot of fishes	Weight of sample	Number of fishes	Mean weight	ml./gm./hr.				
				Procedure 1		Procedure 2		Means
A	4.6	9	0.51	0.370		0.424		
B	5.1	9	0.57	0.341	0.337	0.307	0.320	0.328
C	5.1	9	0.57	0.278		0.200		
D†	3.5	6	0.58	0.358		0.348		
E†	3.8	6	0.63	0.357		0.271		0.314
F	6.6	9	0.73	0.253		0.293		0.273
G	5.1	6	0.85	0.283	0.275	0.212	0.252	0.264
G'*	5.1	6	0.85	0.268		0.293		
H†	5.4	6	0.90	0.309		0.303		
I	5.4	6	0.90	0.306		0.236		
I'*	5.4	6	0.90	0.333	0.322	0.343	0.306	0.314
J	5.5	6	0.92	0.326		0.317		
J'*	5.5	6	0.92	0.334		0.329		
K	7.0	6	1.16	0.153	0.248	0.222	0.230	0.239
L†	7.0	6	1.17	0.343		0.239		
M	7.8	6	1.30	0.277		0.270		
M'*	7.8	6	1.30	0.311	0.244	0.308	0.261	0.252
N	8.0	6	1.33	0.144		0.204		
O	8.7	6	1.45	0.236		0.222		0.229
P	10.0	6	1.67	0.209		0.227		0.218
Q	11.2	6	1.87	0.179		0.205		0.192
R	5.4	1	5.40	0.150		0.111		0.130
S	9.5	1	9.50	0.136		0.153		0.144
	Means		1.56	0.272		0.262		0.267

have been due to the size of the fishes employed. In any case the chief relations here considered are little affected.

(2) *Mormon at Mormon*

It was difficult for practical reasons (distance and time) to leave the Mormon Spring fishes over night in tubes before making the tests of oxygen consumption in their own habitat, as was customary in the

Preston-at-Preston series. Likewise it was found that the usual fifteen minutes after stoppering was too long a period of confinement, considering the high temperature (36° ±) of the water and its low oxygen content.

Two preliminary lots, however, were subjected to this longer test. The values for oxygen consumption for these lots (mean weights 0.76 and 0.67 gm.) were 0.386 and 0.382, ml./gm./hr., respectively. Some of these fishes showed marked signs of asphyxiation during the last few minutes. That respiratory values for partially asphyxiated fishes cannot be regarded as normal is obvious.

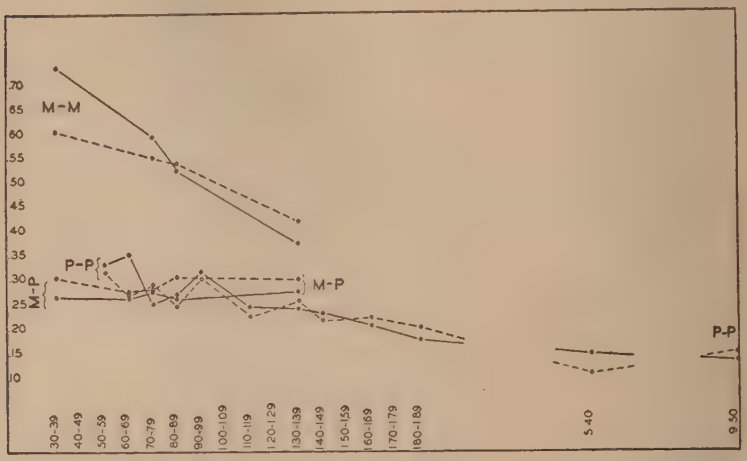

FIG. 3. Comparison of "Preston-at-Preston" series with "Mormon-at-Mormon" and with "Mormon-at-Preston" (1 day). Abscissas = mean weight of fishes in each lot; ordinates = oxygen consumption (ml./gm./hr.). Solid lines connect values obtained by second of above procedures (see legend for Fig. 2); broken lines connect values obtained by the first procedure.

The remaining four tests, limited to ten minutes, are very instructive. In Table II (upper section) it is seen (1) that values for oxygen consumption are far higher in this series than in the "Preston at Preston" series; (2) that these values are inversely correlated with the mean weights of the fishes involved. This correlation is far more evident than in the Preston series, a significant fact which will be discussed shortly. Comparisons between the Mormon-at-Mormon and Preston-at-Preston series may be made by inspection of Fig. 3. That the relations shown here are little affected when we substitute certain more strictly comparable Preston-at-Preston lots, has already been pointed out.

(3) *Preston at Mormon*

It had already been reported by Sumner and Sargent (1940) that Preston Spring fishes speedily die when transferred to Mormon Spring. In the more recent experiments, it was found that the fishes were overcome so rapidly by the heat that no instructive determinations of oxygen consumption were possible.

(4) *Mormon at Preston*

In these experiments, the fishes were transferred from Mormon Spring to Preston Spring in partially insulated bottles. The temperature fell seven or eight degrees in the course of transfer, though this circum-

TABLE II

	Lot of fishes	Weight of sample	Number of fishes	Mean weight	Oxygen consumption (mg. per gm. per hr.) Procedure 1	Procedure 2	Means
Mormon at Mormon	T	3.5	9	0.39	0.739	0.612	0.675
	U	4.4	6	0.73	0.597	0.555	0.571
	V	5.1	6	0.85	0.528	0.544	0.536
	W	8.3	6	1.38	0.382	0.423	0.402
	Means			0.84	0.561	0.533	0.546
Mormon at Preston (2¼ to 4 hours)	T′	Same values			0.422		
	U′				0.374		
	V′				0.328	0.342	0.335
	W′				0.255	0.263	0.259
	Means				0.345		
(1 day)	T″	Same values			0.268	0.308	0.288
	U″				0.256	0.288	0.272
	V″				0.266	0.308	0.287
	W″				0.278	0.304	0.291
	Means				0.267	0.302	0.285

stance would seem to have little bearing on the result. Three different series of these fishes (8 lots, comprising 57 fishes) were thus transferred. The most instructive of these series consisted of the same four lots as were employed in the principal Mormon at Mormon tests. The further history of these fishes is included in Table II, and is portrayed graphically, in part, in Fig. 4. After the test in Mormon Spring, these fishes were left over night in the latter, in the same tubes (screened at the ends). They were then taken in bottles to Preston Spring and tested,

first, after the lapse of 2½ to 4 hours following transfer; next after a day. As regards their performance after this briefer period, we have complete figures only for the second of our procedures in computing oxygen consumption. The mean of these four values is 0.345, which is plainly intermediate between 0.561 (before the transfer) and 0.267 (one day following the transfer).

The significant features to be observed in the behavior of these four lots of fishes at the close of a day following transfer are (1) the great fall in metabolic rate in the cooler water; (2) the inverse correlation be-

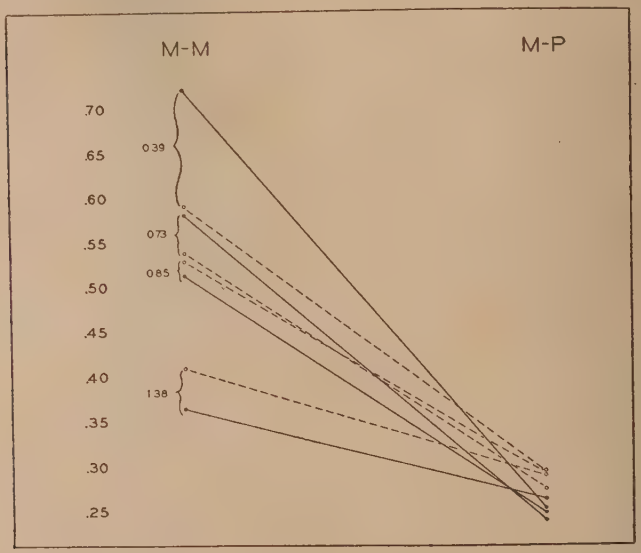

FIG. 4. Rate of oxygen consumption of same group of Mormon Spring fishes: (1) at Mormon Spring; (2) after 1 day at Preston Spring. Ordinates = ml./-gm./hr. Solid and broken lines have same significance as in two preceding figures. Figures opposite brackets denote mean weights of the fishes of these respective lots.

tween the size of the fishes and the rate of this fall, resulting in a marked convergence of the curves for the four size-groups, which come to nearly a common level by the end of 24 hours.

A question of considerable interest is whether the present figures give evidence that the respiratory metabolism of these warm-adapted fishes, when transferred to much cooler water, undergoes any such fall below the rate shown by cool-adapted specimens as had been shown in previous laboratory experiments.

At the close of the day our mean figure for the metabolic rate of six lots of Mormon-at-Preston fishes was 0.284; that for the Preston fishes in their natural habitat being 0.267. The mean weight of the Mormon-at-Preston fishes (0.79 gm.) is, however, much less than that of the Preston-at-Preston fishes (1.56 gms.), a fact which might, perhaps, account for this higher rate. Graphs for these two groups (Fig. 3), in which each is plotted according to weight, make it plain that any possible difference of metabolic rate, in either direction, is small in extent.

It cannot, therefore, be affirmed with any probability that these warm-spring fishes, when transferred to water at 21°, display a rate of oxygen consumption which is lower, at the close of one day, than fishes native to the latter. This conclusion may be regarded as confirming the results of the earlier test with the cyanide method, already referred to.

Two of the eight Mormon-at-Preston lots comprised in our various experiments were kept in the latter spring for two more days.[11] The figures for these two lots (mean weight 0.52 and 0.57 grams) were 0.283 and 0.343. While not very consistent, these figures certainly do not indicate any further fall of metabolic rate.

The convergence of the four pairs of curves in figure 4 is a striking feature of this graph and deserves some comment. At the close of a day in the cool spring, the differences between these four lots, in respect to metabolic rate, have virtually disappeared, or have even been slightly reversed. Thus, the correlation between size and metabolic rate, which is so conspicuous at 36°, has vanished at 21°. It has already been pointed out that this correlation is low in the cool-spring fishes themselves.

A marked divergence of the temperature-O_2-consumption curves for "large" and "small" *Fundulus* is shown by Wells (1935*a*, Figs. 3 and 4). Wells's observations relate to temperatures ranging from 10° to 24°, so that the case is not altogether parallel with our findings on *Crenichthys*, in which we are dealing with water at 21° and 36°, and with no intermediate values. In the case of our warm-spring fishes, too, the correlation actually disappears at the lower temperature. It is plain, however, that the two cases are in essential agreement in one important respect.

That the metabolic rate of organisms declines with age, or conversely, that a higher metabolic rate is to be expected in younger animals, appears to be an accepted fact (Child, 1915; Heilbrunn, 1937). Since fishes are known to grow more or less continuously through life, it is usually a safe assumption to regard relative size as an index of relative age. Our

[11] Some specimens were kept for four and even six days, but these were weighed in the meantime. Since a number of these fishes died as a probable result of the unavoidable drying, the results cannot be regarded as significant.

series of fishes of increasing weight (Table I) may therefore be safely regarded as representing, on the whole, a series of increasing age.

Concerning the correlation between age and the temperature-coefficient, less appears to be known. The majority of workers seem to have reported that this coefficient increased with age (Bělehrádek, 1935). Our figures, on the contrary, as well as those of Wells, certainly indicate a higher temperature coefficient for the *smaller* fishes.

Summary

Fishes (*Crenichthys baileyi*) living in a warm spring at temperatures of 35° to 37° have a far higher rate of oxygen consumption (ml./gm./hr.) than fishes of the same species living in a cool spring (21°). The ratio, when fishes of approximately the same weight are compared, is not far from 2 to 1.

Cool spring fishes, when transferred to the warm spring, die so rapidly that measurements of oxygen consumption are not practicable.

When the converse experiment is performed (transfer of warm-spring fishes to the cool spring), they live in health for some days, and may perhaps do so indefinitely. Changes in their rate of oxygen consumption are as follows: (1) A rapid fall occurs within the first few hours. (2) At the close of a day, the metabolic rate (oxygen consumption) is about the same as that of fishes native to the cool spring. If any difference exists, when specimens of the same size are compared, this is probably slight.

This failure of the metabolic rate of warm-spring fishes to fall below that of cool-spring fishes, when transferred to the latter medium, accords with previous experiments at these same springs in which the cyanide method was used, but it does not accord with experiments performed upon some other species in the laboratory.

A marked inverse correlation exists between size (age) and metabolic rate. This correlation is more marked at high temperatures than at lower ones. Thus the curves for the four size-groups of warm-spring fishes here considered converge strongly during the test in cool water, coming together at nearly a common point by the end of the day. This last fact harmonizes with the fact that the correlation between size and metabolic rate, while observable, was found to be low among the cool spring fishes.

The oxygen consumption of smaller fishes is much more affected by temperature changes than is that of the larger specimens. In other words, the temperature coefficient is higher for the smaller (younger) animals, a fact which does not appear to agree with most results which have been reported for other organisms.

LITERATURE CITED

ALLEE, W. C., AND OESTING, R., 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zoöl.*, **7**: 509–541.

AMERICAN PUBLIC HEALTH ASSOCIATION, 1936. Standard methods for the examination of water and sewage. New York.

BĚLEHRÁDEK, J., 1935. Temperature and living matter. Berlin: *Gebrüder Borntraeger*, 277 pp.

BRUES, C. T., 1928. Studies on the fauna of hot springs in the western United States and the biology of thermophilous animals. *Proc. Amer. Acad. Arts and Sci.*, **63**: 139–228.

CHILD, C. M., 1915. Senescence and rejuvenescence. Chicago: Univ. Chicago Press, 481 pp.

FOX, H. M., 1936. The activity and metabolism of poikilothermal animals in different latitudes. I. *Proc. Zoöl. Soc. London*, pp. 945–955.

——, 1939*a* (same title). III. *Proc. Zoöl. Soc. London, Ser. A*, **108**: 501–505.

——, 1939*b* (same title). V. *Proc. Zoöl. Soc. London, Ser. A*, **109**: 141–156.

FOX, H. M., AND WINGFIELD, C. A., 1937 (same title). II. *Proc. Zoöl. Soc. London*, **107**: 275–282.

HEILBRUNN, L. V., 1937. An outline of general physiology. Philadelphia: *W. B. Saunders*, 603 pp.

HUBBS, C. L., 1932. Studies of the fishes of the order Cyprinodontes. XII. A new genus related to Empetrichthys. *Occasional Papers of the Museum of Zology, University of Michigan*, No. **252**: 1–5.

HUBBS, C. L., AND KUHNE, E. R., 1937. A new fish of the genus Apocope from a Wyoming warm spring. *Occasional papers of the Museum of Zoölogy, University of Michigan*, No. **343**: 1–21.

HUBBS, C. L., AND MILLER, R. R., 1941. Studies of the fishes of the order Cyprinodontes. XVII. Genera and species of the Colorado River system. *Occasional Papers of the Museum of Zoölogy, University of Michigan*, No. **433**: 1–9.

KEYS, A. B., 1931. A study of the selective action of decreased salinity and of asphyxiation on the Pacific killifish, Fundulus parvipinnis. *Bull. Scripps Inst. of Oceanog. (Tech. Ser.)*, **2**: 417–490.

LEINER, M., 1938. Der Physiologie der Fischatmung. Leipzig: *Akademische Verlagsgesellschaft* M.B.H. 134 pp.

SUMNER, F. B., AND DOUDOROFF, P., 1938. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, **74**: 403–429.

——, AND SARGENT, M. C., 1940. Some observations on the physiology of warm spring fishes. *Ecology*, **21**: 45–54.

——, AND WELLS, N. A., 1935. Some relations between respiratory metabolism in fishes and susceptibility to certain anesthetics and lethal agents. *Biol. Bull.*, **69**: 368–378.

WELLS, N. A., 1932. The importance of the time element in the determination of the respiratory metabolism of fishes. *Proc. Nat. Acad. Sci.*, **18**: 580–585.

——, 1935*a*. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. *Physiol. Zoöl.*, **8**: 196–227.

——, 1935*b*. Change in rate of respiratory metabolism in a teleost fish induced by acclimatization to high and low temperature. *Biol. Bull.*, **69**: 361–367.

WINGFIELD, C. A., 1939. The activity and metabolism of poikilothermal animals in different latitudes. IV. *Proc. Zoöl. Soc. London, Ser. A*, **109**: 103–108.

WUNDER, W., 1936. Physiologie der Süsswasserfische Mitteleuropas. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung, 340 pp.

Contribution No. 158

ON THE ENERGY EXCHANGE BETWEEN SEA AND ATMOSPHERE*

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FOREWORD

The present paper is a preliminary report on an investigation of the exchange of heat and water vapor between the sea and atmosphere with particular reference to the regional and seasonal variations in these quantities over the North Atlantic and North Pacific oceans. The *mean annual* evaporation over the oceans according to latitude ranges can be considered fairly well established (Wüst, 1936; Mosby, 1936; McEwen, 1938), but there has been no extensive investigation of the seasonal and intra-latitudinal variations. Since a knowledge of evaporation rates is necessary before computations of the heat exchange between sea surface and atmosphere can be attempted, as a result of lack of these data, very little is known concerning the regional variations in the total energy exchange between sea and atmosphere.

The present study approaches the problem first on the basis of energy considerations such as have successfully been employed by Mosby (loc. cit.), and mean annual evaporation values have been computed for four selected areas in the North Atlantic and North Pacific oceans. The values so derived have then been compared with the evaporation within the same areas computed on the basis of more recent theoretical considerations of the processes of interchange of water vapor within the turbulent layer near the sea surface (Sverdrup, 1937; Montgomery, 1940), when using recently published data on humidities, sea surface temperatures and wind speeds over the oceans. However, the necessary climatic data over the oceans are of doubtful

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„Mass und Zahl behalten
ihre Bedeutung auch in der
modernen Geography."
(N. Krebs)

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accuracy, particularly with reference to humidity, and a comparison of the two methods was necessary in order to determine the statistical adjustment to be applied to these data and further, to establish the validity of the latter method of computation.

By means of the final evaporation equation obtained, seasonal values of evaporation for each five-degree square in the North Atlantic and North Pacific have been computed. In addition, through the use of the regional and seasonal evaporation values so derived, and the Bowen formula (Bowen, 1926), which allows the computation of the ratio between evaporation and heat exchange, charts have been prepared for the same areas showing the total heat and energy exchange between sea surface and atmosphere for all seasons.

I. THE AMOUNT OF HEAT ABSORBED AT THE SEA SURFACE

It is obvious that, for the oceans as a whole, the energy loss from the sea surface during the course of the year must, for all practical purposes, equal the total incoming radiation absorbed at the surface. For small areas of the sea surface it is also evident that the evaporation rate expressed in grams or centimeters of depth may be determined entirely by energy rendered available for that purpose in accordance with the equation:

Notation

E = Evaporation in grams or centimeters.
E_1 = Mean annual evaporation computed by means of the energy equation.
E_2 = Mean annual evaporation computed by means of the theoretical evaporation equation of Sverdrup (uncorrected).
Q_e = Heat of evaporation.
Q_c = Heat exchanged between sea and atmosphere through convection.
Q_a = Total energy exchange between sea and atmosphere $(Q_e + Q_c)$.
Q_t = Total solar energy reaching the sea surface.
Q_b = Total back radiation at $\overline{C}$.
Q_v = Amount of heat added to or removed from the water mass through advective processes.
L_t = Latent heat of vaporization of water $= 594.9 - 0.51t$.
Q_s = Intensity of direct sun radiation upon a surface normal to the sun's rays.
S = Solar constant (1.932 gm. cal. cm^2/min.)
Q_0 = Total incoming radiation upon a horizontal surface with clear sky.

Q_{ab} = Total heat absorbed at the sea surface.
eff Q_b = Effective back radiation with clear sky.
T = Turbidity factor (Linke).
m = Unit dry air mass at $p = 760$.
D = Ratio between direct and total radiation.
C = Percent of cloudiness.
r = Percent of incident solar heat reflected at sea surface.
R = Bowen ratio = Q_c/Q_e.
t_w = Temperature of sea surface.
t_a = Air temperature at height a.
e_w = Vapor pressure at sea surface (0.98 times e at t_w).
e_a = Vapor pressure at height a.
a = Height of observation.
H = Diffusion coefficient (0.235).
p = Pressure.
k_0 = Universal turbulence constant (von Kármán) = 0.4.
W = Friction velocity.
W_a = Velocity at height a.
z_0 = Roughness parameter (Rossby) = 0.6.
d = Thickness of laminar layer.
τ = Tangential stress of wind.
q = Specific humidity.

$$\gamma_a = \frac{k_0}{\ln a/z_0}$$

Γ = Evaporation coefficient (Montgomery).
c_p = Specific heat of air at constant pressure.
ρ = Density.
h = Solar altitude.
A = Eddy conductivity.
$\bar{k}$ = $\bar{Q}_0/\bar{h}$. (Mosby—"Richtungskoeffizient").

$$\underline{E} = \frac{Q_t - rQ_t - Q_b - Q_c - Q_v}{L_t} \qquad \text{I-(1)}$$

where Q_t is the total heat reaching the sea surface, r is the percent reflected, Q_b the heat loss through back radiation to the sky, Q_c the heat exchanged between sea and atmosphere through convective processes, Q_v the quantity of heat added to or removed from the unit water column through advective processes and L_t is the latent heat of vaporization of water at temperature t.

The intensity of the direct sun radiation upon a surface normal to the sun's rays is:

$$Q_s = S_e^{-Ta_m m} \quad \text{g. cal./cm}^2 \text{ min.} \qquad \text{I-(2)}$$

where S is the solar constant (1.932 g. cal./cm min.), e the base of natural logarithms, T the turbidity factor after the definition of Linke (1922), m the unit air mass at $p = 760$ mm. and

$$a_m = 0.128 - 0.054 \log \text{m.} \qquad \text{I-(3)}$$

It is thus only necessary to know the sun's altitude h, the turbidity factor T and, in case of necessity, the air pressure p, in order to determine the intensity of solar radiation at any point on the earth's surface. This value times the sine of $h°$ gives, then, the intensity of the direct radiation on a horizontal surface. But in dealing with the total energy received at the sea surface, Q_0, it is also necessary to take into consideration the diffuse radiation from the sky as well as the direct radiation from the sun. From observations on the *"Maud"* expedition, Mosby (loc. cit.) obtains the following relationship between direct and total radiation:

$$D = 0.88 - \frac{2.4}{h} \qquad \text{I-(4)}$$

and for approximate values of T, obtains:

$$Q_0 = \frac{1}{D} Q_s \cdot \sin h \qquad \text{I-(5)}$$

It has been shown by Mosby, however, that the total incoming radiation on a horizontal surface at sea level can be related to the average solar altitude by the very convenient linear equation:

$$Q_0 = k\bar{h} \text{ g. cal./cm}^2 \text{ min.} \qquad \text{I-(6)}$$

The values of k vary somewhat with latitude within a range of 0.023 to 0.027 due to the varying water vapor in the atmosphere. The product $k\bar{h}$, then, gives the yearly average of the total incoming radiation with a clear sky in the stated units.

Ångstrom (1922), Kimball (1928) and others have determined an approximate relation between daily totals of solar radiation and the average cloudiness, the values for the ratio between radiation with clear sky and totally cloud-covered sky falling within the range 0.22 to 0.30. The differences are largely determined by the nature and thickness of the cloud cover and, perhaps, also by the percentage of cloudiness and solar altitude. Kimball presents 0.29 as the most likely average value. Using this ratio throughout he finds:

$$Q_t = Q_0 \, [0.29 + 0.71 \, (1 - \bar{C}/100)] \qquad \text{I-(7)}$$

where C is the percentage of sky covered by clouds. This allows the determination of the average incoming radiation for any latitude by means of the following formula:

$$Q_t = kh \, [1 - 0.71 \, (C/100)] \qquad \text{I-(8)}$$

This formula, however, determines only that amount of heat incident upon the sea surface; a reduction is necessary to allow for the amount reflected back to the sky. According to Schmidt (1915), the percent reflected, r, varies according to latitude from a minimum of 3.3 at the equator to 8.0 at the poles. These data are given in tabular form by this author and also by Ångstrom (1920) and Mosby (1936). From this it follows that the total solar heat absorbed at the sea surface is:

$$Q_{ab} = Q_t \, (1 - r) \qquad \text{I-(9)}$$

Now, of the total heat absorbed by the water mass, some will be radiated back to space, the amount of which depends upon the absolute temperature of the water surface, the moisture content of the atmosphere and upon the nature and extent of cloud cover. Ångstrom (loc. cit.) has computed values for the back radiation at various temperatures and humidities and the data in the present calculations have been obtained from that source. As suggested, however, the values for the effective back radiation (*eff* Q_b) so obtained must be reduced to allow for the screening effect of any cloud cover present. According to Asklöff (1920), this correction factor amounts to $(1 - 0.0083 \, \bar{C})$. The final value must be further reduced (Ångstrom) by 6% to allow for internal reflection. Thus, the average back radiation is represented by the following formula:

$$Q_b = 0.94 \, [\textit{eff} \; Q_b \; (1 - 0.0083 \, \bar{C})] \qquad \text{I-(10)}$$

The difference $Q_{ab} - Q_b$ represents, therefore, the amount of heat available in the sea for evaporation and for heating the air. It is necessary, as the first step in the computations, to disregard the advection factor. In order to determine the amount of heat used up in the evaporation process it is now only necessary to determine the proportion of energy given off as sensible heat to the atmosphere. All of the investigators of the problem referred to here have assumed a constant value for this proportion but it will be shown that the ratio Q_c/Q_e is highly variable, both with respect to season and with respect to the regional distribution.

II. THE EXCHANGE OF HEAT BETWEEN SEA AND ATMOSPHERE

Mosby (loc. cit.), concerning the exchange of sensible heat between sea surface and atmosphere, states:

> „Um die mittlere Verdunstung bestimmen zu können, müssen wir nunmehr also noch diejenige Wärmemenge kennen, die durch turbulente Konvektion von der Meeresoberfläche zur Atmosphäre verfrachtet wird. Diese Wärmemenge hangt von vielen Verhältnissen ab, in erster Linie von dem Temperaturunterschied Wasser—Luft und von der Windstärke; es ist jedoch heute kaum möglich, sie gehau zu bestimmen."

In his determinations of evaporation from the oceans, Mosby assumes an arbitrary, but reasonable, value of 10% as the proportion of available energy given off as sensible heat to the atmosphere. He appears not to have been acquainted with the work of Bowen (1926), who has established the relation between evaporation and heat exchange at a water surface.

In considering the rates at which heat and water vapor are transported across the lower and upper surfaces of a volume of air above a body of water, Bowen has determined that the ratio, R, of the heat loss by conduction to that by evaporation can be obtained by means of the following formula:

$$R = 0.50 \left(\frac{t_w - t_a}{e_w - e_a} \right) \frac{p}{760} \qquad \text{II-(1)}$$

where t_w and t_a are the temperatures in $°C$ of the water and air, respectively, e_w and e_a are the vapor pressures in millimeters of the water surface and air and p is pressure (mm.). Bowen's derivation of the above formula is too lengthy to be reproduced here but the same equation can be derived quite simply as follows (Sverdrup et al, 1941):

Since the amount of water vapor, F, which is transported upward through the unit surface per second is

$$F = -A \frac{0.623}{p} \frac{de}{dz} \qquad \text{II-(2)}$$

where A is the eddy conductivity, then the amount of latent heat transported as water vapor is

$$Q_e = -L_t A \frac{0.623}{p} \frac{de}{dz} \qquad \text{II-(3)}$$

The amount of sensible heat transported away from the sea surface is

$$Q_c = -c_p A \frac{dt_a}{dz} \qquad \text{II-(4)}$$

where c_p is the specific heat of air at constant pressure. Therefore:

$$R = \frac{Q_c}{Q_e} = \frac{c_p}{L_t} \frac{p}{0.623} \frac{dt_a}{dz} \Big/ \frac{de}{dz} \qquad \text{II-(5)}$$

Setting $c_p = 0.237$ and $L_t = 585$, one obtains

$$R = 0.49 \frac{p}{760} \frac{dt_a}{dz} \Big/ \frac{de}{dz} \qquad \text{II-(6)}$$

Replacing the gradients dt_a/dz and de/dz by $(t_w - t_a)$ and $(e_w - e_a)$, one finds

$$R = 0.49 \frac{p}{760} \left(\frac{t_w - t_a}{e_w - e_a} \right) \qquad \text{II-(7)}$$

Since the present calculations require sea level pressures throughout, obviously the pressure term can be neglected, thus:

$$Q_c = RQ_e = \frac{RQ_a}{1 + R} \qquad \text{II-(8)}$$

$$Q_a - Q_c = \frac{Q_a}{1 + R} = EL_t \qquad \text{II-(9)}$$

and from (7) and (9)

$$E = \left[\frac{Q_a}{1 + 0.49 \left(\frac{t_w - t_a}{e_w - e_a} \right)} \right] \Big/ L_t \qquad \text{II-(10)}$$

III. COMPUTATIONS OF THE MEAN ANNUAL EVAPORATION FOR SELECTED AREAS IN THE NORTH ATLANTIC AND NORTH PACIFIC OCEANS BY MEANS OF ENERGY CONSIDERATIONS

In order to compare the values for evaporation obtained through the use of the energy equations given above with those determined by means of the more recent theoretical considerations of the interchange of water vapor and heat within the turbulent layer of the atmosphere, computations of the mean annual evaporation have been made for four selected areas in the North Atlantic and North Pacific

oceans. Since, as a first step, it is necessary to disregard the advection factor, Q_v, the areas selected were those within which the dia-latitudinal transport of ocean waters is at a minimum. For this reason, coastal areas have been eliminated from consideration. The two latitude ranges chosen were between 40° N. and 45° N. and between 20° N. and 25° N. In the North Atlantic, between 40° N. and 45° N., the evaporation was computed for the area bounded by meridians 20° W. and 50° W. and in the latitude range 20° N.–25° N., by the meridians 25° W. and 65° W. In the North Pacific, between 40° N. and 45° N., the computations have been made for the area bounded by meridians 140° W. and 160° E. and between latitudes 20° N.–25° N., by 130° W. and 140° E.

For the marine climatic data entering into the computations, the writer is indebted to the New Orleans and Washington, D. C., offices of the U. S. Weather Bureau for the loan of manuscript charts presenting recently compiled data on sea surface and air temperature, wet-bulb depression, wind speed and cloudiness over the oceans. Abridged forms of some of these charts appear in the *Atlas of Climatic Charts of the Oceans* (U. S. Weather Bureau, 1938).

The results of the computations are given in Table I. The average annual evaporation in the North Atlantic between latitudes 40° N. and 45° N. proves to be 84 cm/yr.; in the North Pacific, 68 cm/yr. Within the latitude range 20° N.–25° N., the evaporation is 168 cm/yr. in the Atlantic Ocean and 148 cm/yr. in the Pacific Ocean. These results would indicate that evaporation is greater at all latitudes in the North Atlantic than in the North Pacific. The value for the latitude range 40° N.–45° N. in the Atlantic is 16 cm/yr. greater than for the similar latitude range in the Pacific while it is 20 cm/yr. greater for the latitude range 20° N.–25° N. Analysis of the data in Table I indicates that the higher evaporation in the North Atlantic is largely due to the smaller amount of heat lost to the atmosphere rather than to any significant excess of energy received. The mean annual cloudiness within the latitude range 40° N.–45° N. is, however, somewhat higher in the North Pacific than in the North Atlantic which would account for a small proportion of the difference in evaporation at these higher latitudes.

TABLE I—Computations of Evaporation for

	Lat.	*Long.*	k	$\bar{h}°$	$\bar{C}$ (%)	Q_t/Q_o	Qt	r (%)	Q_{ab}
Atlantic	40°N–45°N	25°W–10°W	0.024	14.7	64	0.546	0.193	4.8	0.184
Ocean	20°N–25°N	25°W–65°W	0.023	18.2	46	0.673	0.282	3.8	0.271
Pacific	40°N–45°N	140°W–160°E	0.024	14.7	70	0.506	0.178	4.8	0.169
Ocean	20°N–25°N	130°W–140°E	0.023	18.2	46	0.675	0.283	3.8	0.272

IV. COMPUTATIONS OF THE ANNUAL EVAPORATION BY MEANS OF AVAILABLE DATA ON WIND SPEEDS, HUMIDITIES AND TEMPERATURES OVER THE OCEANS

As an outgrowth of the researches on turbulent mixing in the lower layers of the atmosphere, particularly by Taylor (1932), Prandtl (1935), von Kármán (1935), Rossby (1932; 1935) and Sverdrup (1936), it has been possible to prepare formulae showing evaporation as a function of moisture concentration and wind movement within the turbulent layer. Such formulae have been constructed by Sverdrup (1937) and Montgomery (1940) but since their discussions are presented in readily available publications, no attempt will be made in this paper to enter into the derivations of the equations.

However, the basic assumptions are that the transport of water vapor between sea surface and atmosphere takes place by ordinary diffusion through a shallow boundary layer and by eddy conductivity above this layer. It is also shown that for the unstable atmosphere, i. e., where the potential temperature decreases with height, the eddy conductivity, itself, is a linear function of height and of the roughness character of the sea surface. It is also shown that, under these conditions, the vapor pressure is a function of the logarithm of height. According to Sverdrup:

$$E_2 = \frac{H \dfrac{0.623}{p} \rho \ (e_w - e_a) W}{\dfrac{H}{K_0} \ln \dfrac{a + Z_0}{d + Z_0} + dw} \qquad \text{IV-(1)}$$

where H is the diffusion coefficient, p is the atmospheric pressure, ρ is density, a altitude of observation, k_0 is von Kármán's constant (0.4), W is the friction velocity where

$$W = \sqrt{\frac{\tau}{\rho}} = \frac{k_0}{\ln \dfrac{a + z_0}{z_0}} \quad W_a = \frac{0.165}{\log \dfrac{a \pm z_0}{z_0}} \quad W_a$$

Selected Areas by Means of the Energy Equations

$\bar{t}_w$ (°C)	$\bar{t}_a$ (°C)	$\bar{e}_a$ (mm)	$\bar{e}_w$ (mm)	$eff Q_b$	Q_b	$Q_{ab} - Q_b$	R	Q_c	Q_e	$\bar{L}_t$	E_1 (cm/yr.)	
16.4	16.1	11.9	13.7	0.187	0.083	0.101	0.076	0.007	0.094	586.9	84.0	*Atlantic*
24.8	25.2	19.8	23.0	0.166	0.096	0.175	−0.056	−0.010	0.186	582.3	167.5	*Ocean*
11.1	10.4	8.5	9.8	0.186	0.074	0.095	0.249	0.019	0.076	589.8	67.7	*Pacific*
24.6	23.9	18.6	22.8	0.166	0.097	0.176	0.073	0.012	0.164	582.4	148.2	*Ocean*

W_a is the velocity at level a and z_0 is the roughness parameter (0.6,— Rosby) and

$$dW = \frac{dW_a\ k_0}{\ln \frac{a}{z_0}} = 4.12$$

According to Montgomery:

$$E_2 = \rho k_0\ \gamma_a\ \Gamma\ (q_w - q_a)\ W_a \qquad \text{IV-(2)}$$

where $\gamma_a = \frac{k_0}{\ln \frac{a}{z_0}}$ and Γ is his "evaporation coefficient." Both γ_a and Γ depend upon the hydrodynamic character of the sea surface but the dependence is such that the product $\gamma_a\ \Gamma$ is very nearly constant for either rough or smooth surface. If this is true, equation IV-(2) can be reduced to a formula of the type:

$$E_2 = K\ (e_w - e_a)\ W_a \qquad \text{IV-(3)}$$

Equation IV-(1) also reduces to a formula of this type.

Since $d = \frac{\ln \frac{a}{z_0}\, dW}{k_0\ W_a} = \frac{71.2}{W_a}$

when $z_0 = 0.6$ and $a = 600$

Then, at $W_a = 500$; $\quad d = 0.14$

$W_a = 1500$; $\quad d = 0.05$

Taking $H = 0.235$ and $p = 1000$, from equation IV-(1):

$$E_2 = \frac{0.235 \cdot 0.623 \cdot 1.2 \times 10^{-6} \cdot \frac{0.4}{6.9}\ (e_w - e_a)\ W_a}{0.6 \cdot 6.6 + 4.1}$$

$$= 1.25 \times 10^{-9}\ (e_w - e_a)\ W_a \qquad \text{IV-(4)}$$

where e is in millibars and W_a is in meters per second at a height of six meters.

The 24-hour evaporation in millimeters or decigrams is therefore given by:

$$E_2 = 0.108\ (e_w - e_a)\ W_a \qquad \text{IV-(5)}$$

Equation IV-(1) applies to the adiabatic atmosphere but under conditions of atmospheric stability, the computed values for E_2 will be somewhat too great. However, when considering evaporation over periods of a day or longer, no serious error is introduced as the amounts computed under stable conditions are small when compared with those computed under adiabatic conditions.

Through the use of equation IV-(5), computations of the mean annual evaporation have been made for the same four areas in the North Atlantic and North Pacific. The results are given in Table II. As

TABLE II—Mean Annual Evaporation for Selected Areas Computed by Means of Equation (5), E_2, Compared with the Mean Annual Evaporation, E_1, Computed by Means of the Energy Equations

	Lat.	Long.	E_1 (cm/yr.)	E_2 (cm/yr.)	E_1/E_2	E ($E_2 \times 1.32$) (cm/yr.)
North Atlantic	40°N–45°N	20°W–50°W	84.0	88.3	0.95	116.6
North Atlantic	20°N–25°N	25°W–65°W	167.5	94.2	1.78	124.3
North Pacific	40°N–45°N	140°W–160°E	67.7	55.8	1.21	73.7
North Pacific	20°N–25°N	130°W–140°E	148.2	113.2	1.31	149.4

was expected, the values derived by the latter method are lower than those computed by means of the energy equations. The mean annual evaporation for the four areas proves to be 87.9 cm/yr. when computed by equation IV-(5) and 116.8 cm/yr. when computed by means of the energy equations. Most of this difference can no doubt be assigned to the inaccuracies of the humidity measurements at sea for it would be expected that the mean of a large number of such observations would be too high, the tendency of error in the individual observations being in this direction. This would result, of course, in the computed values of evaporation being too low. However, if these differences resulting from the inadequacy of the observational data are consistent, it is possible to prepare a correction factor to be applied to equation IV-(5) such that the two methods can be brought into agreement. The ratios E_1/E_2 given in the sixth column of Table II, indicate that this factor is of the order of 1.32. Thus equation IV-(5) becomes:

$$E = 0.143\ (e_w - e_a)\ W_a \qquad \text{IV-(6)}$$

It now becomes of interest to examine the nature and causes of the remaining differences $(E_1 - E)$.

In the use of the energy equations under section III for computing E_1, it was necessary to disregard the advection term, Q_v, except to the extent that the sea surface temperature data entered into the computations of Q_b and R. For this reason, it would be expected that the evaporation computed by such methods would be too low at high latitudes and too high at low latitudes. An examination of the data in the last column of Table II shows this to be particularly true in the case of the North Atlantic where the dia-latitudinal transport of surface waters is greater than in the North Pacific. Thus in the North Atlantic, on account of the great northerly transport in the west portion, the greater heat transport is from south to north. In the case of the North Pacific, on the other hand, the northerly transport in the west portion is more nearly balanced by the southerly transport in the east portion. If we set

$$Q_v \cong \Delta Q_{1,2} = (E\ L_t + Q_c) - (E_1 L_t + Q_{c1})$$

then for the Atlantic between latitudes 40° N–45° N., Q_v becomes 20533 g. cal. cm^2/yr. and for the latitude range, 20° N.–25° N., — 23811 g. cal. cm^2/yr. In the Pacific, on the other hand, Q_v is only 444; g. cal. cm^2/yr. for the latitude range 40° N.–45° N. and still shows a slight positive net heat transport between latitudes 20° N.–25° N. of 626 g. cal. cm^2/yr. The ratios of Q_v for North Atlantic and North Pacific indicate that the heat transport into the latitude range 40° N.–45° N. of the Pacific is only 21% as great as the similar transport in the Atlantic within the same latitude range, while in the latitude range 20° N.–25° N. in the Pacific, the inflow of heat is very nearly balanced by the outflow. These results are in line with present information concerning the transport of surface waters in both oceans. It should perhaps be repeated, that since the areas were so chosen as to exclude the coastal sections, the net heat transport for the entire latiude ranges represented would differ from the figures given above.

V. COMPUTATIONS OF THE SEASONAL EVAPORATION, HEAT EXCHANGE AND TOTAL ENERGY EXCHANGE FOR FIVE-DEGREE SQUARES IN THE NORTH PACIFIC AND NORTH ATLANTIC

Through the use of equation IV-(6), it is now possible to compute the mean seasonal evaporation for five-degree squares in the North Atlantic and North Pacific by means of the previously-mentioned data on sea surface and air temperatures, wet-bulb depressions and wind speeds over the oceans. The values for e_w have been obtained

by multiplying the vapor pressures corresponding to the surface temperature t_w, by 0.98 in order to take the effect of salinity into account. The values for e_a have been obtained from the Smithsonian Tables which present vapor pressure as a function of the difference between wet- and dry-bulb temperatures.

Then from the evaporation quantities so determined, and the values of $t_w - t_a$ and $e_w - e_a$, the seasonal and annual values for Q_c and Q_a have been computed for the same areas.

Since the climatic data at hand presented temperatures and wet-bulb depressions in degrees Fahrenheit and the wind speeds in knots, it was convenient to adjust the several equations to the same units such that:

$$E = 0.249\ (e_w - e_a)\ w_a \qquad \text{gms. cm}^2\text{/day} \qquad \text{V-(1)}$$

$$Q_e = 145.4\ (e_w - e_a)\ w_a \qquad \text{gm. cal. cm}^2\text{/day}\ (L = 585) \qquad \text{V-(2)}$$

$$Q_c = 0.01 \left(\frac{t_w - t_a}{e_w - e_a} \right) Q_e \qquad \text{gm. cal. cm}^2\text{/day} \qquad \text{V-(3)}$$

$$Q_a = Q_e + Q_c \qquad \text{gm. cal. cm}^2\text{/day} \qquad \text{V-(4)}$$

in which e is in inches-Hg, t is in degrees Fahrenheit and w_a is in standard knots.

The charts prepared from the mean seasonal and annual values of E and Q_c computed for five-degree squares in the North Pacific and North Atlantic are primarily of interest in the meteorological field which is not only concerned with the amount of heat added to or subtracted from the lower layers of the atmosphere, but also of the amount of latent energy in the form of water vapor which is rendered available for the various meteorological processes. On the other hand, the charts showing the seasonal and regional values of Q_a are of particular interest to the field of oceanography since the loss or gain of energy from the sea surface through either evaporation or conduction is entirely in the form of heat. The charts for E are of interest in this field only to the extent that the evaporation, through its effects upon the surface salinity, serves to alter the distribution of density within the oceans. However, it is not practicable to include the 15 charts necessary to show the seasonal and annual values of E, Q_c and Q_a in the present short paper. It is expected that these will be presented in a later, and more complete, report.

An examination of the charts for E shows that the evaporation in both oceans is nearly everywhere greatest in winter and least in summer; that the regions of greatest evaporation are on the western

sides of the oceans and about the southern margins of the North Atlantic and North Pacific semi-permanent high pressure fields. Evaporation on the eastern sides of the oceans is generally smaller. The absolute maximum evaporation for all oceans occurs in the North Atlantic in winter within the Gulf Stream between latitudes 35° N.–40° N. and about 700 miles east of the Virginia Capes, where the average evaporation is about 1.14 gms. cm^2/day. The maximum value in the North Pacific, 0.94 gms., on the other hand, occurs farther south and nearer the coast within the Kuroshio between latitudes 25° N.–30° N. and approximately 300 miles northeast of Formosa. The evaporation in winter along the eastern sides of both oceans is relatively much smaller, for example, in the North Pacific it is only 0.15 gms. cm^2/day off the coast of British Columbia and Central California and 0.14 gms. off the Southern California Coast. Although the values increase slightly southward, the evaporation is still only 0.18 gms. in the Gulf of Panama. In the North Atlantic off the Irish Coast, the winter value is 0.30 gms. and off the northern coast of Africa, 0.36 gms. The average winter evaporation in the Gulf of Mexico and the Caribbean Sea is of the order of 0.40 gms.

In spring the evaporation decreases considerably along the western sides of the oceans, the influence of the Kuroshio upon evaporation in the North Pacific having almost disappeared while the similar effect of the Gulf Stream in the North Atlantic has become considerably less. The evaporation in the south-central portion of the tropical North Pacific, however, increases slightly during this season and reaches its maximum value. The evaporation along the equator in the eastern North Atlantic also increases slightly but at the same time decreases to the minimum seasonal value within the corresponding region in the North Pacific. In the higher latitudes, the central and western portions of the equatorial regions and along the eastern sides of the oceans, the decrease in evaporation is small.

In summer the evaporation reaches its lowest value over most of the North Pacific and North Atlantic. The values, however, increase slightly during this season within the eastern equatorial regions of both oceans and reach their maximum at this time. The decrease in evaporation within the tropical belts of high evaporation is small. The quantities, nevertheless, remain positive for all areas except in the North Pacific north of latitude 55° and west of 160° W., and in the North Atlantic waters immediately surrounding Labrador, where a slight net condensation is computed for this season. The effect of the Kuroshio in the western North Pacific has now completely disappeared and the computed values of evaporation for the eastern

and western sides of this ocean are of the same order of magnitude. The effects of the Gulf Stream upon evaporation are, however, still in evidence and the evaporation on the west side of the North Atlantic remains greater than in the eastern portions.

In the autumn the evaporation nearly everywhere increases and is greater than during either spring or summer except in the south-central tropical regions in both oceans where the minimum values for these respective areas occur. Evaporation also decreases from its summer maximum in the eastern equatorial regions of both oceans. The effect of the Kuroshio upon evaporation in the North Pacific again becomes evident and evaporation within the Gulf Stream of the North Atlantic now reaches values as high as 0.88 gms. off the Virginia Capes.

Thus, in general, it is shown that the regions of greatest evaporation are those within which the northerly transport of surface waters is greatest, i. e., along the western sides of the oceans within the Kuroshio and Gulf Stream. Conversely, the regions of least evaporation are those of southerly flow or drift, namely, along the eastern sides of the oceans and the extreme northwestern coastal areas. The only exceptions to this general statement appear within the south-central tropical areas of both oceans where, in the northern part of the trade wind region, a relatively high evaporation is associated with the dry descending air currents accompanying the semi-permanent fields of high pressure, and in the eastern equatorial regions, where relatively low values are associated with the northerly flowing ocean currents which in these regions cross the equator resulting in an influx of cold water from the higher latitudes in the Southern Hemisphere.

In the North Atlantic Ocean, the center of the tropical or trade-wind area of maximum evaporation remains nearly stationary during all seasons except autumn, when it quite largely disappears, with its mean position located between latitudes 10° N.–20° N. and approximately one-third the distance between the coasts of South America and Africa. In the North Pacific Ocean, on the other hand, this tropical area of high evaporation is considerably better developed and its center varies in position with the seasons. In winter the maximum evaporation within this area is 0.70 gms. cm^2/day and the center is located between 5° N.–10° N. and 170° W.–175° W. In spring the maximum increases to 0.72 gms. and the center is displaced northeastward to the area between 10° N.–15° N. and 150° W.–155° W. In summer the maximum evaporation in this region decreases to 0.62 gms. and the center is further displaced northeastward to the area between 15° N.–20° N. and 145° W.–150° W. In the autumn, how-

ever, the maximum evaporation within this area falls to its lowest value (0.56 gms.) and the now less well-defined center is displaced southward to the general region on the equator between latitudes 0° N.–5° N. and between 135° W.–150° W. This clockwise migration of the tropical center of high evaporation in the North Pacific appears to correspond to the similar movement of the center of the North Pacific high pressure field.

The average annual values of evaporation for the various latitude ranges are given in Figure 18 and are compared with similar values presented by other investigators. The computed annual values appear to be in excellent agreement with those given by Wüst (1936); the ratio $E/E_{\text{Wüst}}$ for all latitudes being 1.00 for the North Atlantic, 1.06 for the North Pacific and 1.03 for both oceans. The agreement with McEwen's (1938) values is also good but the values are lower than those given by Cherubim (1931). The agreement with Mosby's (1936) values is good for the latitude ranges north of 25°, but his values are considerably higher for the lower latitudes. The greatest departures between the present values and those previously determined by other investigators of the problem, appear within the latitude range 45° N.–50° N., where the previous values given have been somewhat higher, and within the latitude range 35° N.–40° N., where the previous values assigned have been lower. In general, these curves show that evaporation in the North Atlantic is at a maximum within the latitude range 35° N.–40° N. with a well defined secondary maximum between latitudes 15° N.–20° N. In the North Pacific, the maximum annual evaporation occurs between latitudes 20° N.–25° N. with evidence of a slight secondary maximum between latitudes 35° N.–40° N. With regard to the curves shown, it is perhaps well to remark that too much dependence should not be placed upon the values north of latitude 55° since the areas involved in the computations for these regions are rather small.

The isolines for Q_c over the oceans show, roughly, the same configuration as shown for E, these values being at their maximum in winter and along the western sides of the oceans, with the major exception that there exist no tropical areas of maximum heat exchange to coincide with the areas of maximum evaporation which appear associated with the North Pacific and Azores high pressure systems.

The absolute maximum value for Q_c (260 g. cal. cm^2/day) occurs in winter in the North Atlantic in about the same location as the center of maximum evaporation. In the North Pacific, the maximum winter value (240 g. cal. cm^2/day) occurs between 35° N.–40° N. and 150° E.–155° E. with a secondary area of maximum heat exchange northeast

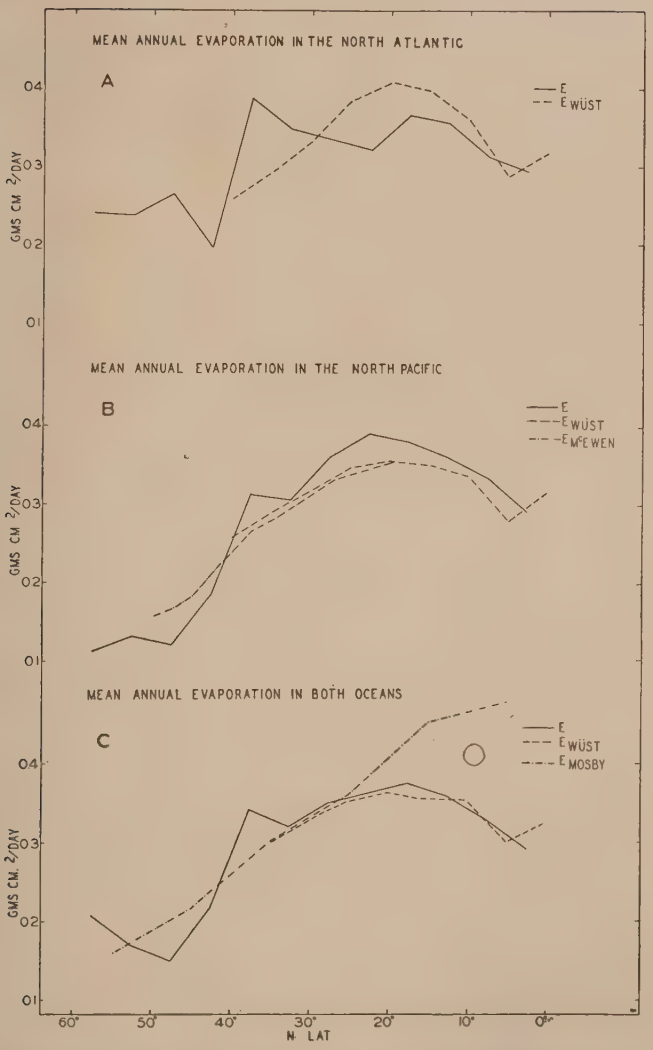

Figure 18. A. The mean annual evaporation in the North Atlantic compared with the values previously determined by Wüst. B. The mean annual evaporation in the North Pacific compared with the values previously determined by Wüst and McEwen. C. The mean annual evaporation in both oceans compared with the values previously determined by Wüst and Mosby for all oceans.

of Formosa coinciding with the area of maximum evaporation. The North Pacific values for Q_c during winter are positive for all regions except for several small areas near the equator. In the North Atlantic, however, the charts show that even during the winter, Q_c is negative for approximately one third the area of that ocean, i. e., roughly southeast of a line drawn northeast from Trinidad, B. W. I., to lat. 40° N., long. 20° W. and thence eastward.

In spring Q_c decreases generally in all areas but still remains positive for most of the North Pacific. Values as high as 116 g. cal. cm²/day occur within the Gulf Stream but Q_c is negative for all that area of the North Atlantic roughly southeast of a line drawn from Panama to lat. 60° N., long. 10° W. A value of — 57 g. cal. cm²/day is computed for the area immediately off the coast of Africa between 15° N. and 20° N. In summer the values for Q_c become insignificant nearly everywhere in the North Pacific except in the southeastern equatorial regions where values as high as 40 g. cal. cm²/day are determined, and are negative everywhere north of latitude 40°, off the California Coast and, surprisingly, also off the Asiatic coasts south to the Philippine Islands. In the North Atlantic in summer, Q_c is negative everywhere except within a narrow zone along the western side extending northeastward from Florida to lat. 40° N., and within the Gulf of Mexico and Caribbean Sea.

In autumn, the values for Q_c increase considerably in both oceans and the distribution is very nearly the same as in spring except that the negative values in the eastern portions of the North Atlantic are not as great.

The mean annual values of Q_c arranged by latitudes are shown in Figure 19A. These curves indicate that the heat exchange between sea and atmosphere per unit area is greater in the North Pacific than in the North Atlantic, particularly south of the latitude range 35° N.–40° N. From minima within the latitude range 45° N.–50° N., Q_c in both oceans increases to the latitude range 35° N.–40° N. and then decreases toward the equator, becoming negative in the North Atlantic south of lat. 25°.

The charts showing the total energy exchange (Q_a) between sea surface and atmosphere are, of course, composite charts of the values of Q_e and Q_c, therefore, in general configuration they are quite similar to the charts for E, except that the west-east gradient in the energy exchange is intensified due to the high values for Q_c over the western sides of the oceans and the low values over the eastern sides. Conspicuous areas of maximum energy exchange over the south-central tropical areas of both oceans coincide with the tropical areas of high

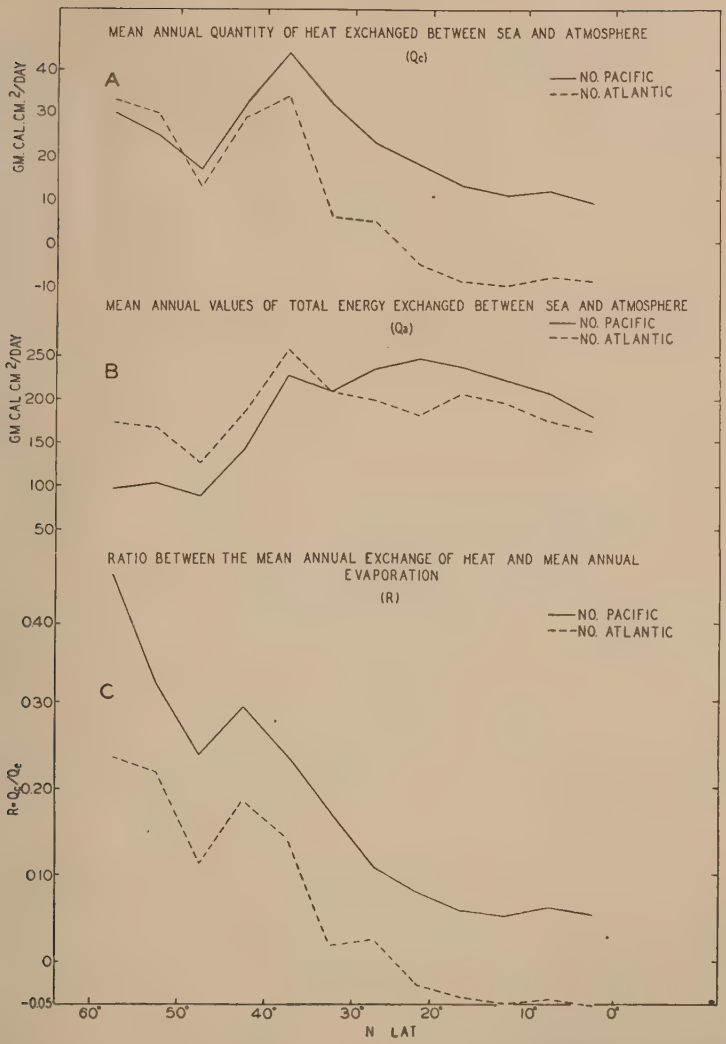

Figure 19. A. Mean annual values for Q_c in the North Atlantic and North Pacific oceans. B. mean annual values for the total energy exchange (Q_a) in the North Atlantic and North Pacific oceans. C. Ratio (R) between the mean annual values for Q_c and Q_e in the North Atlantic and North Pacific oceans.

evaporation as would be expected. In the North Atlantic in winter, Q_a reaches values as high as 937 g. cal. cm²/day within the Gulf Stream off the Virginia Capes. In the North Pacific, the maximum energy exchange occurs in winter within the Kuroshio northeast of Formosa, coinciding with the area of maximum evaporation, where a value of 728 g. cal. cm²/day is computed, with a secondary area of maximum energy exchange (718 g. cal. cm²/day) between latitudes 35° N. and 40° N. approximately 350 miles east of Japan.

The annual values for Q_a arranged by latitudes are given in Figure 19B, which show that the maximum annual energy exchange in the North Atlantic takes place between latitudes 35° N. and 40° N., and in the North Pacific, between latitudes 20° N. and 25° N. with a slight secondary maximum between latitudes 35° N. and 40° N.

Sverdrup (1940) has investigated (by means of the energy equation) the annual variation of the loss of energy from the sea surface in the North Atlantic and North Pacific within two small areas where the necessary observational data were available. In the North Atlantic off the Bay of Biscay at 47° N. and 12° W., he found the energy loss to be greatest in late fall or early winter, becoming zero during summer and with a secondary minimum in February and a secondary maximum in March. These conclusions are in line with the present results for the same region with the exceptions, of course, that neither the secondary minimum nor the secondary maximum could be expected to show in the present calculations. He found the average annual loss of energy in this area to be of the order of 140 g. cal. cm²/day, which is somewhat greater than the present 90 g. cal. average computed for the five degree square immediately west of Sverdrup's point of computation. However, it is to be noted that Sverdrup's calculations have been made for a very small area and one which is quite near the coast, therefore it would be expected that his values would depart somewhat from those computed for a larger area farther westward.

In the North Pacific, Sverdrup has determined, by similar method, the annual variation in the energy loss from the sea surface for a small area within the Kuroshio along the meridian 135° E. between latitudes 31° 50′ N. and 33° 30′ N. He found the energy exchange here also to be at its maximum in winter, the average during December, January and February being approximately 1100 g. cal. cm²/day. This value is considerably higher than the present calculations give for the same general area, i. e., about 600 g. cal. However, again it must be considered that Sverdrup's computations have been made only for a very small area within which the temperature gradients were found to be very steep and should, therefore, give greater values than those computed for the five degree square.

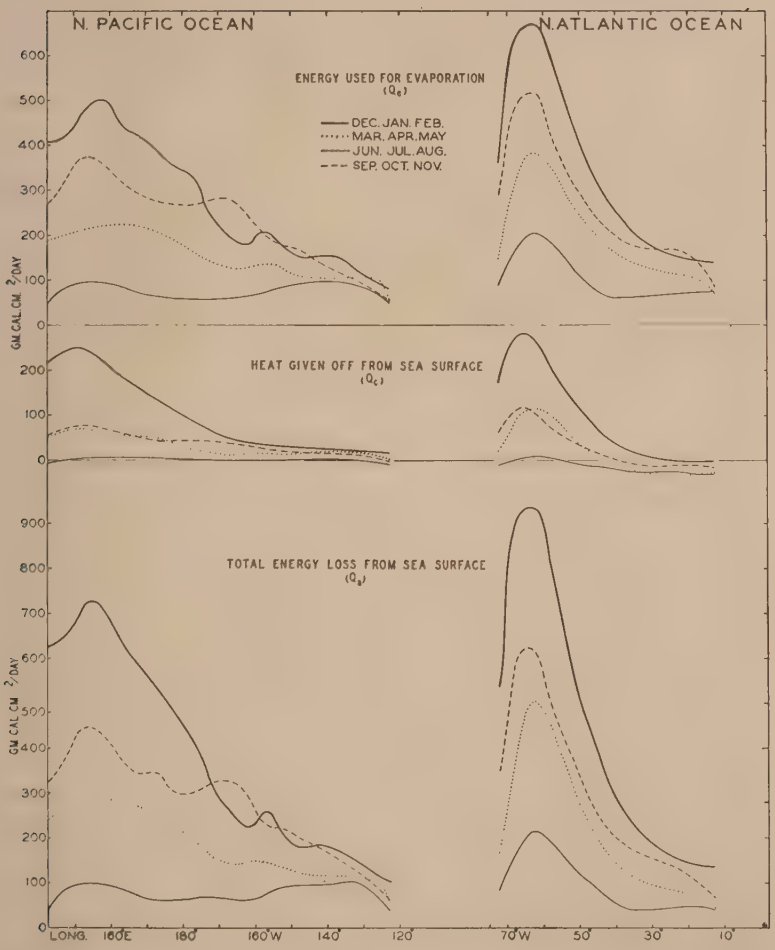

Figure 20. Seasonal variations in evaporation, heat exchange and the total energy exchange within the latitude range 35° N.–40° N. in the North Atlantic and North Pacific oceans.

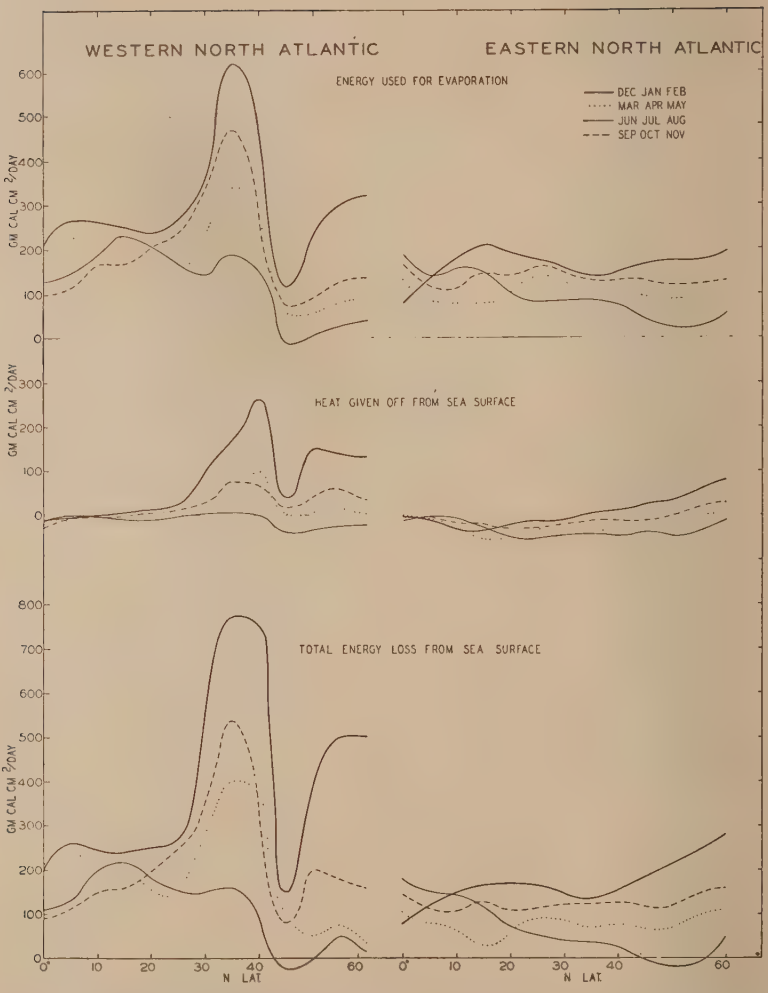

Figure 21. Seasonal variations in evaporation, heat exchange and the total energy exchange according to latitude along the western and eastern sides of the North Atlantic ocean.

The seasonal variations in the quantities Q_e, Q_c and Q_a across the North Pacific and North Atlantic within the latitude range 35° N.–40° N. are shown in Figure 20. These curves serve to illustrate the large seasonal variations in these quantities over the western portions of both oceans, i. e., within the Kuroshio and Gulf Stream, and the relatively much smaller seasonal variations over the eastern portions.

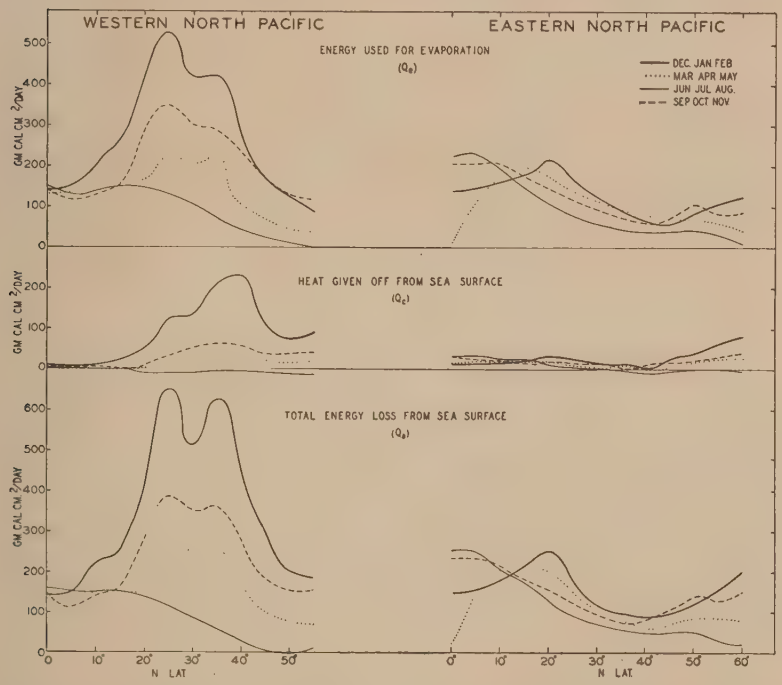

Figure 22. Seasonal variations in evaporation, heat exchange and the total energy exchange according to latitude along the western and eastern sides of the North Pacific ocean.

The seasonal variations in evaporation, heat exchange and the total energy exchange according to latitude along the western and eastern sides of both oceans are illustrated in Figures 21 and 22.[1] These curves show that, in general, the greatest seasonal variations of all

[1] In view of the facts that these north-south cross sections tend to parallel the isolines for Q_e, Q_c and Q_a, and that the selections of the lines of cross section were arbitrary ones, the curves shown in Figures 21 and 22 have been smoothed somewhat in order to eliminate the minor, non-significant variations.

three quantities occur in mid-latitudes along the western sides of the oceans; that the seasonal variations in the equatorial regions in both oceans are small with the exception that in the eastern North Pacific a very decided minimum in spring is shown. It is also indicated that in the western portions of both oceans, the areas of maximum heat exchange (Q_c) occur farther north than do the areas of maximum evaporation (Q_e), the displacement being 15 degrees in the western North Pacific and 5 degrees in the western North Atlantic. The seasonal variations in all quantities within the eastern portions of the oceans are relatively much smaller and, except at the equator as already described, greater in the North Atlantic than in the North Pacific. The seasonal variations at the equator in the western North Pacific and in the eastern North Pacific between latitudes 10° N. and 15° N. and between 25° N. and 45° N. are so small that they can scarcely be considered as significant.

The latitudinal variation in the ratio between the mean annual values for Q_c and Q_e is shown in Figure 19C. These curves indicate that this ratio, R, is higher in the North Pacific at all latitudes than in the North Atlantic and that, in general, the ratios in both oceans decrease from maxima at high latitudes to minima at or very near the equator except for well-defined minima in both oceans between latitudes 45° N. and 50° N. Thus, while the total annual evaporation within the equatorial and tropical regions is high, the total exchange of heat between sea and atmosphere during the year in these areas is small and, conversely, while evaporation at high latitudes is small, the exchange of heat between sea and atmosphere is relatively great. Similarly, it must be concluded that a greater amount of the available energy at the sea surface in the North Pacific is used in heating the atmosphere directly than is the case in the North Atlantic where a greater proportion is used in the evaporation process.

The regional distribution of values for Q_a during the winter and summer seasons is shown in Figures 23A and 23B. The charts illustrated are quite similar in configuration to those for E during the same seasons and also, in a qualitative manner, quite similar to those for Q_c except that in the latter case, as mentioned previously, there exist no maximum areas of heat exchange within the tropical trade-wind region to correspond to those areas of maximum E and Q_a within this belt.

The locations of the principal frontal zones as given by Petterssen (1940) are also shown in Figure 23B and it is interesting to note that these zones correspond quite closely with the zones of maximum energy exchange. However, the lines or zones of frontogenesis (cyclogenesis)

themselves, will coincide more nearly with the zones of the maximum temperature gradient thus, in the western portions of the oceans, the principal frontogenetic areas will be located along the eastern coasts of the two continents northwestward from the mean position of the polar fronts themselves. This, according to Petterssen (loc. cit.), is due to the fact that the zones of frontogenesis will tend to move with the air currents toward the axis of outflow and will assume a mean

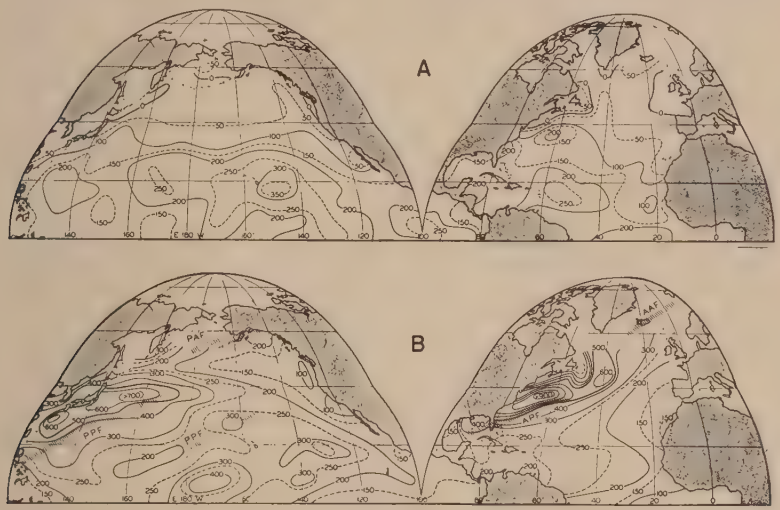

Figure 23. A. Total energy exchange (gm. cal. cm²/day) between sea and atmosphere during summer (June, July, August) in the North Atlantic and North Pacific oceans. B. Total energy exchange (gm. cal. cm²/day) between sea and atmosphere during winter (Dec., Jan., Feb.) in the North Atlantic and North Pacific oceans. The locations of the principal frontal zones in winter (after Petterssen) are shown by the hatched lines.

position near this axis or a trough line in the mean flow. Petterssen's position for the principal frontogenetic zone in the western North Pacific corresponds very closely with the position of the zone of maximum cyclone frequency as determined by Richardson (1936). It is found then, that in both oceans the zones of frontogenesis (cyclogenesis) will in every case lie to the northwestward of a zone of maximum energy exchange and it will be noted (see Figure 23B), in addition, that the major axes of the zones of maximum energy exchange will parallel the principal axes of the frontal zones.

Before concluding the discussion of results, it should be emphasized that the mean seasonal data presented in this report can by no means

be taken as representative of instantaneous conditions over the oceans. It is expected that over given intervals of time of a year or less, the actual values for E, Q_c and Q_a will depart widely from the values given herein, particularly over the western portions of the oceans, and that the positions of the areas of maximum or minimum energy exchange will also vary. Thus over the Kuroshio and Gulf Stream, at the times of outbreak of cold continental air in winter, these values may occasionally be extremely high; conversely, during periods in winter when the polar fronts are located near the coast and the air masses over the neighboring oceans are of southerly origin, and quite warm and moist, the energy exchange in these areas must reach low values. Also, along the eastern shores, the energy exchange in winter may reach high values at the times of outbreaks of dry continental air from the interior. So, just as there are important non-periodic variations in the positions of the principal centers of action in the atmosphere, the polar fronts, etc., with the resulting time or regional changes in humidity, wind, temperature, cloudiness, etc., so must there be corresponding changes in the amount of energy exchanged between sea and atmosphere. In light of such reasoning, it appears that any attempts toward a solution of the problems of long-period weather forecasting must be concerned not only with the dynamics of the atmosphere (and sea) but of the thermodynamical aspects of the problem as well.

SUMMARY AND CONCLUSIONS

While it has long been known that evaporation over the oceans is governed by humidity, sea surface temperature and wind speed, empirical methods used in the past to relate evaporation to these factors have not been successful, largely because the equations either contained unevaluated functions, or were constructed to fit a special set of observations. The multiplicity of such equations, each of only limited use, indicates the difficulty of the evaporation problem. The present investigation, however, offers a method for the proper evaluation of such an equation through a comparison with evaporation computed by means of the energy equations when using long-period climatic data over the oceans. The practicability of the technique has been demonstrated and the following evaporation equation presented:

$$E = 0.143\ (e_w - e_a)\ W_a \quad \text{mm/24 hours.}$$

where the vapor pressure is in millibars and the wind speed in meters per second at the height $a = 6$ meters above the sea surface.

It is further shown that the quantity of heat exchanged between sea and atmosphere can be computed by means of the following equation:

$$Q_c = 0.65\left(\frac{t_w - t_a}{e_w - e_a}\right)Q_e \quad \text{where } p \text{ is const.} = 1000 \text{ mb.}$$

By means of these equations and using available seasonal climatic data over the North Pacific and North Atlantic, computations have been made of the amount of evaporation and of the quantity of heat and total energy exchanged between sea and atmosphere within each five-degree square during the several seasons. Some of the more important conclusions drawn from these computations are that:

1. Evaporation over the North Atlantic and North Pacific is nearly everywhere greatest in winter, least in summer and somewhat greater in autumn than during spring. The only major exceptions are on the equator in the eastern portions of both oceans, where the maximum occurs in summer, and in the south-central tropical North Pacific, where there is a slight maximum in spring.

2. The regions of greatest evaporation are those of northerly flowing ocean currents, i. e., along the western sides of the oceans within the Kuroshio and Gulf Stream. The only exceptions in this case occur within the tropical trade wind belts of both oceans where a high evaporation is associated with the dry descending air currents in these regions, and in the eastern equatorial belts of both oceans where a low evaporation is associated with the northerly flowing cold currents from the Southern Hemisphere which cross the equator in these regions. In winter, evaporation reaches values as high as 1.14 gms. cm^2/day in the Gulf Stream and 0.94 gms. cm^2/day within the Kuroshio. In mid-latitudes along the eastern sides of the North Pacific, the winter evaporation is of the order of 0.15 gms. cm^2/day, and in the eastern North Atlantic, 0.25 gms. cm^2/day.

3. The zones of maximum annual evaporation in the North Atlantic occur farther north than in the North Pacific, viz., within the latitude range 35° N.–40° N. in the North Atlantic and within the latitude range 20° N.–25° N. in the North Pacific.

4. The seasonal variations of evaporation are greatest in mid-latitudes and along the western sides of the oceans and somewhat greater in the North Atlantic than in the North Pacific.

5. Evaporation is a positive quantity for all oceans except during summer for several small areas at high latitudes in the northwest portions.

6. The distribution of values representing the amount of sensible heat exchanged between sea and atmosphere is similar to the dis-

tribution of values for evaporation with the major exception that there exist no tropical areas of maximum heat exchange to coincide with the tropical areas of high evaporation. Also, except during summer, the center of the area of maximum heat exchange in each ocean is situated farther north than the centers of maximum evaporation, the displacement being 15° in the North Pacific and 5° in the North Atlantic.

7. The values for Q_c are generally positive in the North Pacific except for large areas in summer; in the North Atlantic, these quantities are negative for large areas during all seasons, even in winter.

8. The ratio between the amount of heat exchanged between sea and atmosphere and evaporation is greatest at high latitudes and decreases toward the equator; is greater in the North Pacific at all latitudes than in the North Atlantic.

9. The total evaporation and energy exchange is greater in the North Atlantic than in the North Pacific at the higher latitudes (above 30° N.–35° N.) but the reverse is true at lower latitudes.

10. And, as suggested by 7 and 8, the total amount of sensible heat exchanged between sea and atmosphere is greater in the North Pacific than in the North Atlantic at all latitudes below 50° N.

11. It is shown that the locations of the principal frontal zones over the oceans correspond closely with the areas of maximum energy exchange. It is also shown that the principal frontogenctic regions in every case lie to the northwestward of a zone of maximum energy exchange and that their axes are parallel to the major axes of the zones of maximum energy exchange.

12. It is suggested that for every major meteorological change over the oceans there must be a corresponding change in the energy relationships between sea and atmosphere. Thus, it would seem that any attempts toward solution of long-period weather problems must be concerned with thermodynamical as well as dynamical factors.

ACKNOWLEDGMENTS

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LITERATURE

Ångstrom, A.

1920. Application of heat radiation measurements to the problems of the evaporation from lakes and the heat convection at their surfaces. Geogr. Ann., Heft 3, 16 pp., 1920.

1922. Note on the relation between time of sunshine and cloudiness in Stockholm, 1908–20. Arkiv für matematik, Astronomi och Fisik, Band 17, No. 15, Stockholm, 1920.

Asklöff, S.

1920. Über den Zusammenhang zwischen der nächtlichen Wärmeausstrahlung. der Bewölkung und der Wolkenart. Geogr. Ann. No. 3, 1920.

Bowen, I. S.

1926. The ratio of heat losses by conduction and by evaporation from any water surface. Phys. Rev. 27; 779–787, 1926.

Cherubim, R.

1931. Über Verdunstungsmessung auf See. Ann. der Hydro u. Marit. Meteor. 59, Heft. 9, pp. 325–334.

Cummings, N. W. and Richardson, Burt

1927. Evaporation from lakes. Phys. Rev., 30: pp. 527–534, 1927.

Kármán, Th. von

1935. Some aspects of the turbulence problem. Fourth Internatl. Cong. Applied Mechanics Proc. 1934: pp. 54–91, 1935.

Kimball, H.

1928. Amount of solar radiation that reaches the surface of the earth on the land and on the sea. Mo. Wea. Rev., 56: pp. 393–398, 1928.

Linke, Franz, and Boda, Karl

1922. Vorschläge zur Berechnung des Trübungsgrades der Atmosphäre aus den Messungen der Intensität der Sonnenstrahlung. Met. Zeit. 39, p. 161.

McEwen, George M.

1930. Results of evaporation studies conducted at the Scripps Institution of Oceanography and the California Institute of Technology. Bull. Scripps. Inst., Tech. Ser. Vol. 2., pp. 401–415, 1930.

1938. Some energy relations between the sea surface and the atmosphere. Sears. Found. Jour. Mar. Res. Vol. 1, No. 3, pp. 217–238, 1938.

Montgomery, R. B.

1940. Observations of vertical humidity distribution above the ocean surface and their relation to evaporation. Papers in Physical Oceanography and Meteorology, Mass. Inst. of Technol. and Woods Hole Oceanographic Inst., Vol. III, No. 4, 30 pp.

Mosby, H.

1936. Verdunstung und Strahlung auf dem Meere. Ann. der Hydrog. u. Marit. Meteor. Vol. 54, pp. 281–286.

PETTERSSEN, SVERRE

1940. Weather analysis and forecasting. First Ed. McGraw-Hill Book Co., Inc., New York, 1940, 503 pp.

PRANDTL, L.

1935. The mechanics of viscous fluids. *In* Durand, W. F., ed. Aerodynamic Theory, Vol. 3, pp. 34–207, Berlin.

RICHARDSON, R. W.

1936. Winter air-mass convergence over the North Pacific. Mo. Wea. Rev. Vol. 64, No. 6, pp. 199–203, 1936.

ROSSBY, C.-G.

1932. A generalization of the theory of the mixing length with applications to atmospheric and oceanic turbulence. Mass. Inst. Technol. Met. Papers, 1 (4): 36 pp.

——— and MONTGOMERY, R. B.

1935. The layer of frictional influence in wind and ocean currents. Papers in Physical Oceanography and Meteorology, Mass. Inst. of Technol. and Woods Hole Oceanographic Inst. 3 (3): 101 pp.

SCHMIDT, W.

1915. Strahlung und Verdunstung an freien Wasserflächen. Ann. der Hydrog. u. Marit. Meteor. Heft. 3 u. 4, 1915.

SVERDRUP, H. U.

1936. The eddy conductivity of the air over a smooth snow field. Geofysiske Publikasjoner, 11 (7): 69 pp.

1936. Das maritime Verdunstungsproblem. Ann. der Hydrog. u. Marit. Meteor. Vol. 64, pp. 41–47.

1937. On the evaporation from the oceans. Sears Found. Jour. Mar. Res. Vol. 1, No. 1, pp. 3–14.

1940. On the annual and diurnal variation of the evaporation from the oceans. Sears Found. Jour. Mar. Res. Vol. III, No. 2, pp. 93–104.

——— JOHNSON, M. W. and FLEMING, R. H.

1941. The oceans: their physics, chemistry and general biology. Prentice-Hall, Inc. 1941. New York. (in press.)

TAYLOR, G. I.

1932. Transport of vorticity and heat through fluids in turbulent motion. Proc. Roy Soc. (A). 135, pp. 685–705.

U. S. WEATHER BUREAU

1938. Atlas of climatic charts of the oceans. W. B. No. 1247, 1938.

WÜST, G.

1936. Oberflächensalzgehalt, Verdunstung, und Niederschlag auf dem Weltmeere. Landerkundliche Forschung, Festschrift Norbert Krebs, pp. 347–359, 1936.

Contribution No. 159

MICROORGANISMS IN MARINE AIR

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MICROORGANISMS IN MARINE AIR[1]

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THE salubrious properties of marine air have been attributed to its freedom from dust and terrigenous microorganisms, including bacteria, spores and pollens. While in general marine air contains fewer microorganisms than that of continental or terrestrial origin, it is by no means germ free. Wherever careful examinations have been made, regardless of the horizontal or vertical distance from land, microorganisms have been found in the air over the ocean.

Nearly three-fourths of the earth's surface, or 71 per cent, is covered with water most of which is ocean. On this basis the air of the world is predominantly marine. However, as pointed out by Showalter (1939), the origin and movement of an air mass must be taken into consideration in classifying it as marine or continental. Marine air masses often move far inland before assuming continental characteristics, particularly at great elevations, and relatively pure continental air masses sometimes are carried far over the sea. Unfortunately most studies on aerobiology have been conducted on or over land with little or no attention being given to whether the air masses under investigation were of marine or continental origin. Moreover, the few exploratory studies which have been made of the air over the ocean have failed to take into consideration the specific cultural requirements of marine microorganisms and thereby have excluded them.

The microbial population of the air itself might offer a clue to the origin of the air masses if enough data were available. For example, the presence of microinsects or pollens indicates a terrigenous history. The same is true of air in which mold spores are abundant because virtually all molds with the possible exception of a few marine forms (Sparrow 1936) which are readily recognizable are indigenous to the lithosphere only. Similarly the diatoms, blue-green algae and protozoa found in the air are usually terrigenous.

While one soon learns to recognize many of them by their typical cultural characteristics, distinguishing marine bacteria from terrestrial species is attended by difficulties because morphologically, physiologically and culturally they are much alike. Since the microbial population of air is predominately bacterial, the characteristics of the bacteria have received considerable attention.

CHARACTERISTICS OF MARINE BACTERIA

Although there is a continual interchange of bacteria between the land and the sea by wind and water, there appear to be relatively few bacteria which are common to both habitats. This is indicated by the small percentage of bacteria found in the sea at places that are remote from possibilities of terrigenous contamination which give rise to colonies when planted on nutrient media prepared with fresh water, and by the small percentage of fresh water or terrestrial species which form colonies on nutrient sea water media. Terrestrial bacteria can be acclimatized to grow in sea water media and massive inocula of laboratory cultures usually reproduce when transplanted on sea water media but those occurring in nature are fastidious in their salinity requirements, as is illustrated by the data summarized in Table I.

It will be observed that, with the exception of the soil samples collected near the sea, from 80 to 90 per cent of the bacteria from different sources can be classified statistically as marine or terrestrial upon a basis of their ability to grow on sea water media. The results are in agreement with

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 159.

the observations of Berkeley (1919), Korinek (1926), Lipman (1926) and others whose findings have been summarized by ZoBell (1941a).

Coming from an environment, the ocean, most of which is colder than 5° C (Sverdrup *et al.* 1942), it is not surprising to find that in general marine bacteria are more sensitive to high temperatures than their terrestrial cousins. ZoBell and Conn (1940) report that few marine bacteria grow at temperatures exceeding 25° C. Some have thermal death points at 30° to 40° C and may be killed by brief exposure to the plating temperature of agar (42° C). This characteristic is not especially useful in the identification of marine bacteria, but unless it is taken into account many marine bacteria may be excluded by conventional cultural procedures.

It is significant to note in Table I that, whereas in soil collected considerable distance from the sea there were only 18 per cent as many bacteria which grew in sea water medium as in fresh water medium, in soil collected near the sea there were nearly half as many (48 per cent) which grew in sea water as in fresh water medium. This is attributed primarily to the continual aerial transport of large numbers of marine bacteria from the sea to the land, although the higher salt content of the soil adjacent to the sea is partly responsible for the fact that many of the bacteria in the salt-water sprayed soil have become acclimatized to grow at higher salinities. During periods of excessive turbulence along the littoral zone appreciable quantities of salt water may be blown on the land by onshore winds. According to Jacobs (1937), Cauer (1938), Wright (1940) and others, sea salt is characteristic of marine air masses. Yoshimura (1936) attributes the salt content and halophilic bacterial population of certain fresh water lakes near the sea to onshore breezes.

TABLE I

Relative Numbers of Bacteria from Marine and Terrestrial Sources which Developed in Nutrient Media Prepared with Fresh Water or Sea Water

(The ratio of the sea water (SW) to the fresh water (FW) counts is also given.)

Source of inocula	No. of samples analyzed	Nutrient sea water medium	Nutrient fresh water medium	Ratio SW : FW
Raw sea water	31	100	9	11.11
Marine mud	18	100	19	5.26
Soil (near sea)	7	48	100	0.48
Soil (inland)	16	18	100	0.18
Sewage	14	12	100	0.12
River water	8	11	100	0.11
Mouth microflora	5	9	100	0.09

How Marine Bacteria Get into the Air

There is evidence that bacteria are being carried continually from the sea into the air along with droplets of water. The transfer is most intense along rugged coasts when waves are dashed against the rocks or along gently sloping shores where the incoming swell breaks into foam. The transfer is most extensive in the open sea where strong winds often break the ridges of waves (Wright 1940).

Since with a steady or increasing wind the forward motion of the waves is always less than the velocity of the wind, water droplets are blown into the air from their crests. When the height of the waves approaches one-seventh of their wave length, the crests break and the sea becomes covered with white caps (Sverdrup *et al.* 1942). Under these conditions one can actually see water being carried into the air. According to Montgomery (1940) the effect of spray increases the evaporation from the sea by 100 per cent at a wind velocity of about 15 miles per hour, a conclusion which further emphasizes the extent to which water droplets are carried into the air from the sea.

Large droplets fall back into the sea almost at once but the small ones may be lifted to great elevations by advection and convection currents, and carried away by the wind to regions far removed from the

place of origin. The almost universal distribution of sea salt in the air makes this manifest (Jacobs 1937; Wright 1940). Woodcock (1940) cites the soaring of sea birds as evidence of the extent and magnitude of convection currents over the ocean.

According to Blair (1937) droplets of water exceeding 1.0 mm in diameter may be conveyed several thousand feet above sea level by convection, orographic uplift and particularly by vertical motion caused by converging winds (Fig. 1). In the absence of any lifting force whatever a water particle 0.5 mm in diameter could be carried about a mile by a steady wind having a velocity of 10 miles per hour before it could fall a mile. Larger droplets would not fall much faster because they break into smaller ones when they attain a velocity of 800 meters per second (Blair 1937). As shown by the data in Table II, a droplet 0.5 mm in diameter falls through the air at a velocity of 760 cm per second. Smaller droplets fall considerably slower as expressed by Stoke's law of falling bodies, and particles of all sizes would be carried farther by winds of greater velocity. Their rate of fall would be retarded, stopped or reversed by rising currents of air. Since the air is always in turbulent motion, suspended particles are actually carried many times farther than indicated in Table II.

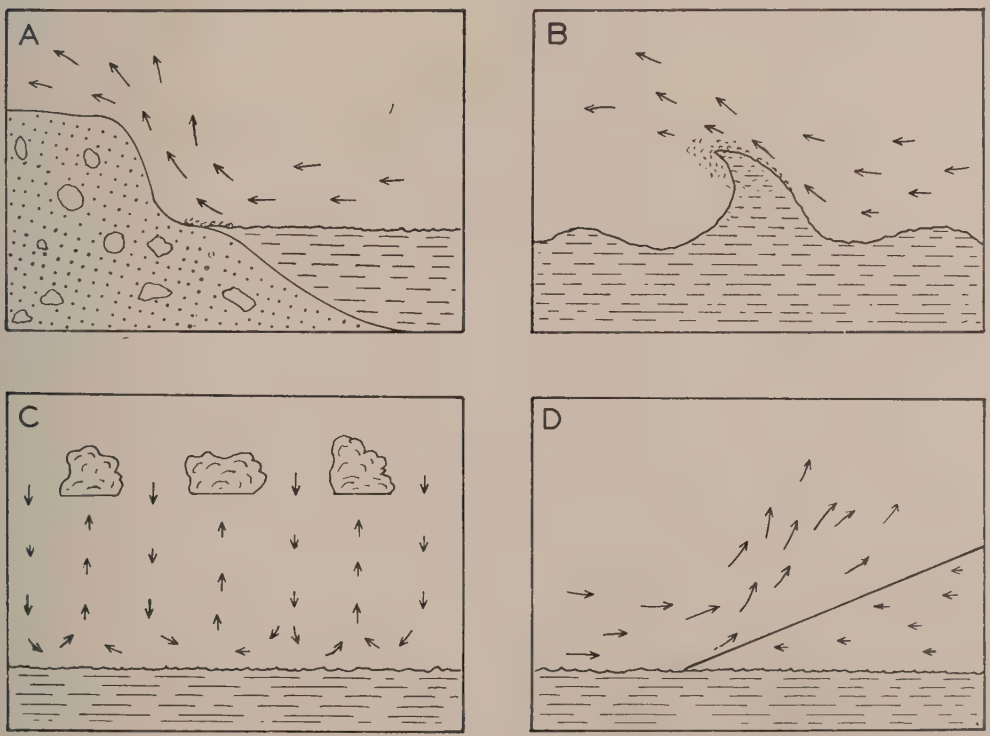

FIG. 1. Principal ways in which bacteria in sea water are carried into the atmosphere: (A) orographic uplift, (B) spray from crests of waves some of which are 20 to 30 feet high, (C) convection and (D) convergence of light warm air mass and heavy cold air front.

These theoretical considerations are based upon a steady state in which the size of the droplets would not change by evaporation or condensation of water. The effect of humidity upon the rate of evaporation and fall of droplets of different sizes is discussed by Wells (1934).

Assuming that they have a density of 1.0, spherical shaped bacteria 1.0 micron in diameter would be carried over 900 miles

TABLE II

RATE OF FALL OF WATER DROPLETS THROUGH THE AIR AND THE MINIMUM* DISTANCE THEY WOULD BE CARRIED BY A STEADY WIND HAVING A VELOCITY OF 10 MILES PER HOUR BEFORE THEY WOULD REACH THE EARTH FROM A HEIGHT OF 10 METERS IN THE ABSENCE OF TURBULENCE

Diameter of droplet	Rate of fall of droplet	Distance carried by wind
Microns	*cm/sec**	*Miles*
1	0.003	926.0
2	0.012	232.0
5	0.076	36.6
10	0.305	9.2
50	7.6	0.36
100	30.5	0.09
200	122.0	0.02
500	760.0	0.0036

* Air rising at this velocity would prevent the descent of particles of the stated size and at greater upward air velocities the particles would be carried to higher elevations. Therefore droplets might be and probably are carried much farther than the stated distance because air is usually turbulent to some degree.

by a steady wind having a velocity of 10 miles per hour before they would reach the earth from a height of 10 meters. In turbulent air (and some degree of turbulence nearly always prevails) the bacteria would be suspended almost indefinitely unless carried to earth by precipitation. Mischustin (1926) and Proctor (1935) report the presence of living microorganisms at elevations ranging from 10,000 to 20,000 feet. Bacteria as well as mold spores were found 70,000 feet above the earth in the stratosphere flight of the National Geographic Society (Armstrong 1936). As pointed out by Jacobs (1940), the horizontal distance over which microorganisms can be transported is almost limitless and is largely determined by their ability to survive the atmospheric environment.

AERIAL TRANSPORT OF MARINE BACTERIA

The extent to which marine bacteria are transported by sea breezes is shown by the observations of ZoBell and Mathews (1936). They exposed plates of nutrient agar on top of the super-structure of a building at the Scripps Institution about 100 feet above sea level and 600 feet from shore (Fig. 2). The plates of media were exposed to the air for an hour after which the lids were replaced and the plates were incubated for 10 days at 22° C. From the representative results which are given in Table III it will be observed that onshore

TABLE III

NUMBER OF BACTERIA WHICH DEVELOPED ON SEA WATER AND FRESH WATER MEDIA FOLLOWING EXPOSURE TO ONSHORE AND OFFSHORE BREEZES AT LA JOLLA

Direction of wind	Velocity of wind	Sea water medium	Fresh water medium	Ratio SW : FW
	m.p.h.			
Onshore	5.4	138	34	4.06
Onshore	3.1	95	27	3.51
Onshore	7.4	206	39	5.28
Onshore	1.9	62	23	2.96
Onshore	3.0	154	43	3.58
Offshore	1.1	112	240	0.47
Offshore	0.9	52	97	0.53
Offshore	2.6	64	294	0.22
Offshore	4.5	179	262	0.68

breezes carried more marine than terrestrial bacteria (as indicated by their ability to grow in nutrient sea water agar) while the reverse was true for offshore breezes. Moreover, the number of bacteria which developed on the plates were found to be more or less proportional to the wind velocity.

The data recorded in Table III merely illustrate the bacterial carrying capacity of sea breezes and are not to be interpreted as depicting differences in the bacterial populations of air masses of marine or terrestrial origin because the direction of the wind in a given location does not necessarily indicate the origin of an air mass.

It so happened, though, that the air masses were exclusively marine, part of which was moving seaward after a temporary excursion over the land along the coast as shown by meteorological data supplied by the United States Weather Bureau. This may account for the fact that the offshore breezes carried a larger percentage of marine bacteria than ordinarily found in soil or other terrestrial environments, although it is more probable that many marine or microbial population of marine air although it must be recognized that earlier in its life history the air has passed over land.

Many complications are encountered when an attempt is made to ascertain how far inland marine bacteria are carried by marine air. While traveling over the land, air masses of marine origin gradually assume a continental character, including the microbial population unless the air mass is

FIG. 2. Plates of nutritive media were exposed on top of the building in the center, on the structure at the end of the 1000-foot pier and on the crosstree three feet from the mainmast sixty feet above the deck of the *E. W. Scripps*.

halophilic bacteria were wafted up from the coastal soil back towards the sea.

The prevailing winds in the Southern California area are from the sea where the air masses are essentially polar or tropical maritime of the purest sort (Byers 1934; Jacobs 1940). The air masses usually present over the region may have passed over several thousand miles of sea surface since contact with land. Thus this region offers excellent opportunities for studying the traveling above the zone of terrestrial turbulence, and it is not easy to obtain samples for analysis at such an elevation. As the air moves inland many bacteria fall to earth probably in water droplets, and more and more terrestrial bacteria will find their way into the air. This is illustrated by the data in Table IV which shows the average number of marine and terrestrial bacteria found in an onshore breeze at different distances from the sea under comparable

TABLE IV

AVERAGE NUMBER OF BACTERIA WHICH DEVELOPED ON SEA WATER AND FRESH WATER NUTRIENT AGAR EXPOSED TO AIR DURING AN ONSHORE BREEZE

Distance from sea wall	Sea water medium	Fresh water medium	Ratio SW : FW
Meters			
0	461	43	10.70
100	548	74	7.40
200	263	109	2.41
400	174	157	1.18
800	128	183	0.71
1600	49	106	0.46

conditions. All plates were exposed six feet above the ground for two hours, and beyond the influence of any vertical obstruction which might serve as a source of contamination. The number of mold spores found in the air, as well as the ratio of molds to bacteria, also usually increases with distance from the sea.

When the ratio of marine bacteria to terrestrial bacteria is less than 1 : 1, it becomes very difficult to detect the presence of the former. Only by detailed physiological, cultural and morphological tests is it possible to prove that certain bacteria are marine species, although many colonies can be recognized as typically marine or typically terrestrial. As pointed out by Jacobs (1940), the chances of finding even one marine bacterium in the air is remote except relatively near the sea because earthy materials such as dust with its accompanying bacteria, are much more likely to be wafted into the atmosphere than is sea water. The latter rarely contains more than a few hundred bacteria per cc at the most, while there are often millions of bacteria associated with an equivalent volume of earthy materials. Moreover, greater thermal and topographical irregularities on the earth's surface which alter both the velocity and turbulence of air currents give the air over the land a much greater lifting and transporting power than that over the sea.

Nevertheless ZoBell and Mathews (1936) demonstrated the presence of appreciable numbers of marine bacteria in a sea breeze having a velocity of 5 to 12 miles per hour on Laguna Mountain, 80 miles inland, a few hours after a rainstorm. At this time nearly twice as many bacteria developed on exposed plates of sea water medium as on fresh water medium. The same day on the observation tower of Mt. Woodson, which is 20 miles inland and has an elevation of 2890 feet, the ratio of the sea water to the fresh water plate count was 2.06, thereby indicating a preponderance of marine bacteria in the air. However, it should be emphasized that ordinarily terrestrial bacteria predominate in the air over the land except in the wake of spray-laden sea breezes. It was only on this one occasion after the rain had probably cleared the atmosphere and settled the dust that the number of marine bacteria exceeded the number of terrestrial species for any appreciable distance inland.

MICROBIAL CONTENT OF PRECIPITATION

Terrestrial microorganisms predominate in rain water. Samples collected aseptically atop S. I. O. buildings and on the end of the pier, which extends 1000 feet into the sea, have been found to contain many more bacteria which grow on fresh water media than on sea water media. Characteristic of continental air many mold spores occur in the rain water, molds usually exceeding the number of bacteria. This generalization also applies to rain water collected when the prevailing wind was onshore and the storms were of known marine origin. The rain water contained from 10 to 150 microorganisms per cc, which formed colonies on fresh water medium and about one-tenth as many which grew on sea water medium. Very few of the latter were identified as marine species.

Under conditions which would prevent sea spray from being carried into the air in the immediate vicinity (and this possibility is largely precluded during a downpour), one would expect to find fewer than one marine bacterium per cc of rain water on the average. This conclusion is based

upon the sea salt content of rain water which is around 5 mg per liter (Jacobs 1937) as compared to 35,000 mg of sea salt per liter of sea water. Therefore only one part of sea water is carried bodily into the atmosphere for each 7000 parts of water evaporated from the oceans. Since surface sea water rarely contains more than a few hundred bacteria per cc, it follows that even should all of the bacteria in the droplets of sea water which are carried into the atmosphere survive, when diluted 1:7000 there would be fewer than one marine bacterium per cc of rain water.

Based upon the sea salt content of atmospheric water of which 89,000 cubic miles are evaporated from the ocean per year (McEwen 1936), around 12.7 cubic miles of sea water is carried into the atmosphere per year exclusive of the much greater volume of larger droplets of sea spray which momentarily falls back into the sea or along the coast. If the droplets of sea water carried into the atmosphere contain an average of 100 bacteria per cc and all of them survive, there would be enough bacteria in 12.7 cubic miles of sea water to provide only one bacterium per square centimeter of the earth's surface per year. This may explain why so few marine bacteria are found on Petri dishes (area 60 cm^2) following their exposure to the air beyond localities where it is laden with sea spray.

The large numbers of microorganisms found in rain water by certain investigators are undoubtedly almost exclusively terrestrial species. Inestimably large numbers of bacteria are carried along with dust into the atmosphere (Lange and Jochimsen 1928) many of which are not killed by the light, heat and dryness of arid desert air (Brown 1930).

McLean (1918) recovered microorganisms from falling snow on Adelie Land, Antarctica, which he believed were carried from distant continents by air currents. Two gram-positive cocci, a gram-negative sporulating rod and three yeasts were isolated. *Bacillus mesentericus, B. subtilis, B. cereus, B. megatherium* and other well-known terrestrial species belonging to the genus *Bacillus, Achromobacterium, Flavobacterium* or *Micrococcus* were about the only bacteria found by Darling and Siple (1941) in freshly fallen snow samples from Little America, the Queen Maude Mountains and Marie Byrd Land. The same general types of bacteria were found on plates of nutrient media exposed to the air. Although they admit that migrating birds, such as terns, gulls and petrels, might carry bacteria to the region, Darling and Siple are convinced that most of the bacteria are air-borne from the continents. Unfortunately selective media were not used to ascertain if there were any marine bacteria in the air and snow of the region.

Bacteria in Air over Ocean

There have been very few surveys made of the microbial content of air over the ocean. The early studies of Certes (1884) on the Talisman Expedition and of Fischer (1886), in which the conclusion was reached that marine air is virtually germ-free together with the lack of opportunities for investigating the air over the ocean, have discouraged extensive research. According to Fischer ocean air beyond the influence of land contains no bacteria except under special circumstances. He recognized that the spray or "sea dust" from turbulent waters might carry bacteria into the air for short distances, but this was regarded as purely a localized phenomenon. He made several hundred analyses of the air over the ocean between Kiel, Plymouth, Cape Verde Islands, Barbados, the Azores and Trinidad on the cruise of the *S. M. S. Molke.*

Fischer's studies indicated that the microbial content of air over the ocean is primarily a function of the distance from land, the direction and strength of the wind and precipitation. When the prevailing direction of the wind was onshore, bacteria were rarely found over the ocean more than 10 or 20 miles from land and ordinarily they were not carried more than 70 to 120 miles from land by strong off-

shore winds. However, he admits the possibility of bacteria being carried to sea along with terrigenous dust which travels the world over. He stresses the cleansing action of precipitation. Corroborative evidence on the paucity of bacteria in the air over the ocean at considerable distance from land has been recorded by Levin (1899), Schmidt and Weis (1902), Gazert (1902, 1903), Pirie (1913) and Hesse (1914).

Unfortunately the early investigators failed to recognize that marine bacteria differ in their salinity and temperature requirements from fresh water bacteria. It is entirely possible that the use of sea water media would have revealed the presence of 10 to 20 times as many bacteria over the ocean as were detected by fresh water media. Significantly Fischer (1886) usually found many more bacteria developed on thin layers of gelatin in tubes through which air was aspirated than on plates of nutrient agar. He attributed this to the greater tendency of bacteria-laden droplets of sea water carried into the air from the crests of waves to be carried into the gelatin coated tubes. However, it is not impossible that the salt water carried to the thin layers of gelatin made it possible for the fastidious marine bacteria to form colonies.

ZoBell and Mathews (1936) found appreciable numbers of marine bacteria in the air 20 feet above the water level at all stations occupied by the *Scripps* at distances ranging from 5 to 130 miles from land. On two cruises during offshore foehn winds many more terrestrial than marine bacteria were found in the air, and the mold count exceeded that of bacteria. However, on the other cruises when the prevailing direction of the winds was onshore and meteorological data indicated that the air masses were predominantly if not exclusively marine, there were from 0.63 to 4.37 times as many bacteria which developed on plates of sea water media as on corresponding fresh water media.

Most of the bacteria which are recovered from marine air on sea water media are new and undescribed species. Typical of marine microflora (ZoBell and Feltham 1934) gram-negative bacilli predominate. Very few cocci are found and I have not observed an air-borne vibrio or spirilla on any of the sea water plates, although between 2 and 3 per cent of the bacteria in the sea are spirilla. Less than half of the bacteria recovered from marine air are spore formers. Gazert (1912) and Hesse (1914) found virtually no cocci or spore-forming bacteria in marine air. This should be contrasted with the abundance of gram-positive rods, spore-formers and cocci which together with mold spores normally characterize continental air.

A pink yeast which is widely distributed in sea water shows up on plates of sea water media exposed to marine air with a high degree of frequency. As far as I know this pink yeast has never been named nor identified although several investigators including Fischer (1894) have mentioned it. McLean (1918) recovered two pink yeasts from marine air over Antarctica.

Of the 100 different cultures of bacteria isolated at random by Rittenberg (1940) from plates exposed to marine air only 15 proved to be species listed by Bergey (1939). These were isolated from plates of fresh water media. Most of the 15 listed species were the same as those found in the upper atmosphere by Proctor (1934, 1935) and Proctor and Parker (1938). They were mostly species which are commonly found in soil and air including six species of Bacillus, three species of Staphylococcus, two species of Micrococcus and one species each of Flavobacterium, Achromobacter and Sarcina. The majority of the new species isolated from marine air belong to the family Bacteriaceae, Pseudomonadaceae, Bacillaceae or Micrococcaceae, with the abundance in the order named.

Little is known concerning the potential pathogenicity of air-borne bacteria over the sea. Since marine bacteria are not pathogenic for man, if pathogens occur in marine air, they must come from the land. No coliform bacteria have been found in the

sea beyond the zone of terrigenous contamination (ZoBell 1941) and an extensive search has failed to detect any in the air (Proctor 1934). While *Escherichia coli, Eberthella typhosa, Vibio comma, Salmonella* and other enteric bacteria may be found in polluted sea water, they are never found in the open ocean nor have they been detected on plates of E. M. B. agar exposed to the air. One would not expect to find plant pathogens in marine air unless they come from the land (*cf.* Christensen 1942).

Reitana and Morselli (1938) found bacteria in the air at all stations where observations were made 2 to 9 kilometers from the coast over the Mediterranean sea. They were mostly common terrestrial species including 13 different species of Micrococcus, 8 species of Flavobacterium, 5 species of Bacillus, 4 species of Rhodoccus and 3 species of Staphylococcus. Their experimental procedures precluded the possibility of detecting marine bacteria since they used fresh water media exclusively and they incubated their cultures at 37° C, which is several degrees higher than the maximum temperature tolerated by most marine bacteria (ZoBell and Conn 1940). Giudice (1939), who likewise disregarded the specific cultural requirements of marine bacteria, found from 12 to 280 bacteria per cubic meter of air in samples collected from thirty stations on the Red Sea and the Mediterranean Sea. Very few of these were chromogens. He observed no relationship between the wind direction or wind velocity and the number of bacteria in the air.

The observations summarized in the preceding paragraph were made only a few feet above the water level. Both the numbers and kinds of microorganisms found in the air over the ocean at greater elevations might be vastly different.

Mold Spores in Marine Air

The number of mold spores found in the air over the ocean generally decreases with distance from land. This is illustrated by the data of Rittenberg (1940) summarized in Table V. Attention is also directed to

TABLE V

Number of Bacteria and Molds which Developed on Plates of Sea Water and Fresh Water Media Exposed for One Hour at Different Distances from Land

Distance from land in nautical miles	Sea water medium		Fresh water medium		Ratio SW : FW	
	Bacteria	Molds	Bacteria	Molds	Bacteria	Molds
0– 10	45	115	20	200	2.25	0.57
10–150	48	79	13	69	3.69	1.14
150–400	71	20	39	36	1.82	0.56

the fact that in this series of experiments the number of bacteria found in the air actually increased with distance from land while the number of molds decreased. As a rule the number of bacteria as well as of as molds decreases with distance from land, not only because many more terrestrial than marine bacteria find they way into the air, but also because the spray from breaking waves along an open coastline promotes the transport of marine bacteria into the air.

Unlike bacteria most molds grow almost equally well on either sea water or fresh water nutrient medium. Moreover, unlike most terrestrial bacteria mold spores survive for long periods of time in the sea. Although only a few highly specialized molds normally inhabit the sea (Sparrow 1936, 1937), a few molds nearly always develop on appropriate nutrient media inoculated with sea water or marine mud (ZoBell and Feltham 1934). While their abundance in the sea decreases with distance from land, the significance of their presence in the air over the sea is problematical. It is most likely that they have been blown out directly from land although they might have been carried back into the air after a temporary sojourn in the sea.

It is quite evident that all of the mold spores in the air over the ocean are terrestrial species and their abundance in the air is probably indicative of the origin and movement of the air mass in which they are found. All of the fungi isolated from

marine air by Rittenberg (1940) proved to be common terrestrial species, Penicillium (18), Hormodendron (15), Sporotrichum (7), Cladosporium (7), Cephalosporium (7), Alternaria (6), Stemphylium (3), Actinomycete (2) and Macrosporium (2) occurring with the percentage frequency indicated by the numbers in parentheses. Most of the molds recovered from marine air far from land by Fischer (1886) were common terrestrial species of Penicillium, Aspergillus and Mucor. Additional information on air-borne fungus spores is given by Christensen (1942).

Pollens in Marine Air

Erdtman (1938) made a quantitative study of pollens in the atmosphere while crossing the Atlantic Ocean from Gothenburg, Sweden, to New York on the *M. S. Drottningholm* in the spring of 1937. He found an average of from 0.7 to 18.0 pollen grains per cubic meter of air at the different stations. These figures may be contrasted with the average of 18,000 pollen grains per cubic meter of air near Stockholm. In general, the absolute number of pollen grains in marine air was related to the distance from land, the direction and velocity of the wind and the flowering time of plants. Pollens of trees, shrubs, herbs, grains and grasses were found right in the middle of the ocean several hundred miles from the nearest land.

Meier and Lindbergh (1935) found pollens, as well as fungus spores, algae, diatoms and insect wings, in Arctic air several hundred miles from land and at an elevation exceeding 3000 feet. They make no mention of bacteria. Besides pollen, Darwin (1878) found several kinds of microorganisms in air-borne dust in the Atlantic Ocean 600 miles from land.

Pollen surveys over the ocean at different elevations made by air-lines and other agencies during recent months show that while pollens are widely distributed, their abundance drops off sharply with distance from land and in the face of marine air fronts. For further information on the aerial dissemination of pollens the reader is referred to Durham (1942).

The Atmosphere as an Environment for Microorganisms

The number of living microorganisms found in the atmosphere is largely a function of the vertical and horizontal movement of air, precipitation and the ability of the microorganisms to survive the great extremes of temperature, humidity and solar radiations to which they are subjected. The temperature of microorganisms suspended in the air is very nearly the same as that of the surrounding air due to their small heat capacity (Jacobs 1940). Therefore it is doubtful if they are subjected to excessively high temperatures in the air. Certain microorganisms probably succumb to the sub-zero temperatures which usually prevail at higher elevations and higher latitudes, although there are some which survive at temperatures approaching absolute zero (ZoBell 1934).

Experiments with marine bacteria indicate that they are killed by ultra-violet radiations just as readily as fresh-water or terrestrial species. Tests on pure cultures of marine bacteria which have been recovered from the air considerable distance from the sea show that they are not endowed with any extraordinary resistivity to ultraviolet radiations. When shallow layers of sea water suspensions of the isolated cultures were exposed to sunlight the bacteria were rapidly though not quantitatively killed, a property which is characteristic of marine bacteria in general (ZoBell and McEwen 1935). Similarly, most of them were killed when suspensions streaked on agar plates were exposed to sunlight.

In view of these observations it should be reiterated that the bacteria on which the tests were made were recovered from the air by exposing open agar plates for different periods of time. Under these conditions the bacteria which settled on the nutrient agar were exposed to sunlight for sufficient time to kill most bacteria under the aforementioned test conditions. Exposure of plates during cloudy weather, at night and with opaque shields between the plates and the sun indicated that very few, if any, of the bacteria which settled on the nutrient agar from the air were killed by

sunlight. This is illustrated by the data in Table VI.

TABLE VI

AVERAGE NUMBER OF BACTERIA WHICH DEVELOPED ON PLATES OF SEA WATER AFTER DIFFERENT PERIODS OF EXPOSURE TO THE AIR IN THE PRESENCE OF BRIGHT SUNLIGHT AND IN THE DARK

Time of exposure	Exposed in sunlight	Exposed in darkness
minutes	*colonies*	*colonies*
5	15	11
10	29	28
20	48	40
30	82	71
60	156	134
90	227	183
120	290	253
240	471	426

In these experiments plates of nutrient agar were exposed to the air for different periods of time under the midday sun or after dark. The exposed plates were then incubated and the colonies counted. It will be observed that the number of bacterial colonies which developed on the plates increases more or less arithmetically with period of exposure until there are so many that crowding may tend to restrict the number which develop regardless of whether they were exposed to sunlight or not.

While there are many factors which influence the rate of settling of bacteria from the air, it is believed that the results indicate that the air-borne parent cells are relatively resistant to sunlight. Their resistivity may be due to extrinsic conditions or it is possible that certain cells are inherently exceptionally resistant. Be this as it may the progeny of the cells exhibited no exceptional resistivity to ultraviolet radiations under similar test conditions. Sharp (1940) found a small percentage of the individuals of a culture of *Pseudomonas aeruginosa* to survive dosages of ultraviolet radiation which are ordinarily lethal. However, the resistivity did not persist in sub-culture progeny. Sharp's results suggest that some bacteria are not hit or that non-fatal single- or even multiple-hits are not necessarily due to individual differences in cell resistance.

Microorganisms in the atmosphere which survive partial desiccation may be rendered non-susceptible to ultraviolet radiations by virtue of their reduced water content. Marine air masses may favor the survival of bacteria due to the hygroscopicity of sea salt. Enough sea spray is carried into the air to increase perspicuously the salt content of rain water falling from marine air masses (Wright 1940). Jacobs (1937) calculated that the mass of sea salt averages 0.41 mg per cubic meter of marine air. Since sea salt is hygroscopic, the air must be extremely dry to evaporate all the water. The salt surrounding the bacteria may keep their water content low. Thus the bacteria in droplets of sea water may be protected from total desiccation. Bacteria themselves function as condensation nuclei and hence are generally surrounded by a film of water the thickness of which will be largely a function of the relative humidity of the atmosphere. According to Wright (1940) the average size of water droplets surrounding sea salt or other condensation nuclei in the atmosphere is very little larger than bacteria of average size.

Droplets of water this size suspended in the air may have a greater tendency to deflect ultraviolet radiations than larger drops, and hence may function as a sanctuary for the bacteria therein. It is beyond the scope of this paper to discuss the factors which influence the bactericidal effect of ultraviolet radiations except to point out that there is a difference of opinion among workers in the field concerning the effect of humidity. Rentschler (1940), for example, finds that high relative humidity of the air does not increase the resistivity of air-borne bacteria, while Wells (1940) and Whisler (1940) maintain that ultraviolet radiations are 10 to 20 times more germicidal in dry air than in humid air. These antithetical conclusions suggest that the experimental conditions have not been identical. It is not impossible that the size of the water droplets, their movement, the position of the bacteria relative

to the droplets, the nature, size and number of condensation nuclei, and other conditions besides merely the humidity are important factors.

Irrespective of whether the survival of bacteria in the air is due to extrinsic or intrinsic conditions, air-borne bacteria appear to be remarkably resistant. Viable bacteria have been recovered from the air at distances from their native habitats which indicate that they have been subjected to intense sunlight and other adverse conditions of the atmospheric environment for considerable periods of time. For example, the marine bacteria recovered by ZoBell and Mathews (1936) from the air 80 miles from the seashore during a sea breeze ranging in velocity from 5 to 12 miles per hour had undoubtedly been in the air for at least seven hours, and probably much longer.

There are recorded instances of terrigenous bacteria being recovered from the air over the ocean several days' or weeks' journey from land. Indications are that the terrigenous bacteria recovered from Antarctic air by Ekelöf (1903), McLean (1918) and Darling and Siple (1941) must have been in the air for a good many days, and the same applies to those isolated by Certes (1884) on the Talisman expedition. There is no way of knowing just how long the bacteria recovered at great elevations by Mischustin (1926), Proctor (1935) and Armstrong (1936) had been in the air but it was probably much longer than the time ordinarily required for direct sunlight to kill bacteria.

Summary

A preponderance of bacteria over other kinds of microorganisms is usually characteristic of marine air of the purest sort, although it also contains large numbers of mold fungi along with other types of microorganisms which have been carried over the sea from land by air currents.

Differences in their salinity requirements make it possible to distinguish marine bacteria from terrestrial or fresh water forms in 80 to 90 per cent of the cases.

The mechanism by which bacteria are carried into the air with droplets of sea water is discussed. It is estimated that an average of 12.7 cubic miles of sea water is annually carried into the atmosphere as water droplets which may contain bacteria.

Most of the air-borne bacteria from the sea are gram-negative non-sporulating rods belonging to the family Bacteriaceae with smaller numbers of Pseudomonadaceae, Bacillaceae and others, whereas Bacillaceae and Micrococcaceae along with mold spores usually predominate in terrestrial air-masses.

Pollen grains occur in air over the ocean several hundred miles from land, their abundance being inversely proportional to the distance from land.

Marine bacteria are carried considerable distances inland by air-currents but they soon become lost among the more abundant terrestrial ones.

Bacteria occur in the air over the ocean at all elevations and distances from land where careful tests have been made for their presence. They also occur in rain and snow presumably far removed from their place of origin. There are no instances on record of pathogenic bacteria being found in marine air.

Many of the bacteria suspended in the air survive the adversities of the atmospheric environment for prolonged periods although paradoxically under laboratory conditions the bacteria isolated from the air are quickly killed by exposure to sunlight.

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References Cited

Armstrong, H. G. 1936. Bacteria in the stratosphere. *Jour. Aviation Med.*, 7: 55–56.

BENECKE, W. 1933. Bakteriologie des Meeres. Abderhalden's Handb. der. biol. Arbeitsmethoden. Abt. IX, Lfg., 404: 717–872.

BERGEY, D. H. 1939. Manual of Determinative Bacteriology. William & Wilkins Co., Baltimore, 1032 pp.

BERKELEY, C. 1919. Study of Marine Bacteria. Strait of Georgia, B.C. *Trans. Roy. Soc. Canada, 3rd Ser. Sec.* 5: 15–43.

BLAIR, T. A. 1937. Weather Elements. Prentice-Hall, Inc., New York, 72: 322–323.

BROWN, J. G. 1930. Living Micro-organism in the Air of the Arid Southwest. *Science,* 72:322–323.

BYERS, H. R. 1934. The Air Masses of the North Pacific. *Bull. Scripps Inst. Oceanogr., Tech. Ser.* 3: 311–354.

CAUER, H. 1938. Einiges über den Einfluss des Meeres auf den Chemismus der Luft. *Balneologe,* 5: 409–415.

CERTES, A. 1884. Sur la culture, a l'abri des germes atmosphérique, des eaux et des sediments rapportes par les expéditions du Travailleur et du Talisman. *Compt. rend. Acad. Sci.* 98: 690–793.

CHRISTENSEN, J. J. 1942. Long Distance Dissemination of Plant Pathogens. [Problems in Aerobiology, A.A.A.S. Publ.] This volume, page 78.

Committee on Apparatus in Aerobiology. 1941. Techniques for Appraising Air-borne Populations of Microorganisms, Pollen and Insects. *Phytopath.,* 31: 201–225.

DARLING, C. A. and SIPLE, P. A. 1941. Bacteria of Antarctica. *Jour. Bact.,* 42: 83–98.

DARWIN, C. 1878. An Account of the Fine Dust which often Falls on Vessels in the Atlantic Ocean. Memoir Descriptive of the Northern Atlantic Ocean by A. G. Finley, London, p. 831.

DURHAM, O. C. 1941. Air-borne fungus spores. Problems in Aerobiology, A.A.A.S. Publ. This volume, page 32.

EKELÖF, E. 1903. Bakteriologische Studien während der schwedischen Südpolar Expedition, 1901–1903. *Wissenschaftl. Erg. der schwed. Südpolar Expedition,* 4: 22–46.

ERDTMAN, G. 1938. Pollen Grains Recovered from the Atmosphere over the Atlantic. *Acta Horti Gotoburgensis, Meddelanden fran Gotesborgs Botaniska Trädgard,* 12: 185–196.

FISCHER, B. 1886. Bakteriologische Untersuchungen auf einer Reise nach Westindien. *Zeitschr. f. Hyg.,* 1: 421–464.

———. 1894. Die Bakterien des Meeres nach den Untersuchungen der Planktonexpedition. Ergeb. d. Planktonexped. d. Humboldt-Stiftung. Kiel and Leipzig.

GAZERT, H. 1902. Die Deutsche Südpolar-Expedition. V. Bakteriologische Untersuchungen. *Veroff. des Inst. f. Meereskunde,* 1: 53–55.

———. 1903. Die Deutsche Südpolar-Expedition. VI. Bakteriologische Bericht. *Veroff. des Inst. f. Meereskunde,* 5: 154–160.

———. 1912. Untersuchungen über Meeresbakterien und ihren Einfluss auf den Stoffwechsel im Meere. Deutsche Südpolar Expedition 1901–3, 7: 235.

GIUDICE, A. 1939. Esame batteriologico dell'aria sul Mediterraneo e sul Mar Rosso. *Ann. di Med. Nav. e Colon.,* 45: 520–523.

HESSE, E. 1914. Bakteriologische Untersuchungen auf einer Fahrt nach Island Spitzbergen und Norwegen im Juli 1913. *Centralbl. f. Bakt. I Abt.,* 72: 454–477.

JACOBS, W. C. 1937. Preliminary Report on a Study of Atmospheric Chlorides. *Month. Weather Rev.,* 65: 147–151.

———. 1940. A Discussion of Physical Factors Governing the Distribution of Microorganisms in the Atmosphere. *Jour. Mar. Res.,* 2: 218–224.

KORINEK, J. 1926. Über Süsswasserbakterien im Meere. *Centralbl. f. Bakt., II Abt.,* 66: 500–505.

LANGE, B. and JOCHIMSEN, E. 1928. Verbreitung von Mikroorganismen in Staubform. *Zeitschr. f. Hyg.,* 108: 66–87.

LIPMAN, C. B. 1926. The Concentration of Sea Water as Affecting its Bacteria Population. *Jour. Bact.,* 12: 311–313.

MCEWEN, G. F. 1936. The Water Cycle between the Ocean, Atmosphere and Land. *U. S. Naval Inst. Proc.,* 62: 956–959.

MCLEAN, A. L. 1918. Bacteria of Ice and Snow of Antarctica. *Nature,* 102: 35–39.

MEIER, F. C. and LINDBERG, C. A. 1935. Collecting Micro-organisms from the Arctic Atmosphere. *Scient. Monthly,* 40: 5–20.

MISCHUSTIN, E. 1926. Mikroflora der hoheren Luftschichten. *Centralbl. f. Bakt., II Abt.,* 67: 347.

MONTGOMERY, R. B. 1940. Observations of Vertical Humidity Distribution above the Ocean Surface and their Relation to Evaporation. *Papers in Physical Oceanogr. and Meteorol.,* 7: 1–30.

PIRIE, J. H. 1913. Notes on Antarctic Bacteriology. The Scott. Oceanograph. Lab., Edinburgh. Abstracted in *Centralbl. f. Bakt., I Abt.,* 58: 263–264.

PROCTOR, B. E. 1934. The Microbiology of the Upper Air. I. *Proc. Amer. Acad. Arts and Sci.,* 69: 315–340.

———. 1935. The Microbiology of the Upper Air. II. *Jour. Bact.,* 30: 363–375.

PROCTOR, B. E. and PARKER, B. W. 1938. The Microbiology of the Upper Air. III. An Improved Apparatus and Technique for Upper Air Investigations. *Jour. Bact.,* 36: 175–184.

REITANO, U. and MORSELLI, G. 1938. Ricerche sui germi dell'aria marina a diversa distanza dalla costa. *Ann. di Med. Nav. e Colon.,* 44: 530–534.

RENTSCHLER, H. C. 1940. Bactericidal Ultraviolet Radiation and its Uses. *Ann. Convention of Illuminating Engineering Society,* 34: 1–16.

RITTENBERG, S. C. 1940. Investigations on the Microbiology of Marine Air. *Jour. Mar. Res.,* 2: 208–217.

SCHMIDT, E. W. and WEIS, F. 1902. Die Bakterien. Jena.

SHARP, D. G. 1940. The Effects of Ultraviolet Light on Bacteria Suspended in Air. *Jour. Bact.*, 39: 535–547.

SHOWALTER, A. K. 1939. Further Studies on American Air-mass Properties. *Month. Weather Rev.*, 67: 204–218.

SPARROW, F. K. 1936. Biological Observations on the Marine Fungi of Woods Hole Waters. *Biol. Bull.*, 70: 236–263.

————. 1937. The Occurrence of Saprophytic Fungi in Marine Muds. *Biol. Bull.*, 73: 242–248.

SVERDRUP, H. U., JOHNSON, M. W. and FLEMING, R. H. 1942. The Oceans: Their Physics, Chemistry and General Biology. Prentice-Hall, Inc., New York, 990 pp.

WELLS, W. F. 1934. On Air-Borne Infection. II. Droplets and Droplet Nuclei. *Amer. Jour. Hyg.*, 20: 611–618.

————. 1940. Bactericidal Irradiation of Air. Part I. Physical Factors. *Jour. Franklin Inst.*, 220: 347–372.

WHISLER, B. A. 1940. The Efficacy of Ultraviolet Light Sources in Killing Bacteria Suspended in Air. *Iowa State Coll. Jour. Sci.*, 14: 215–231.

WOODCOCK, A. H. 1940. Convection and Soaring over the Open Ocean. *Jour. Mar. Res.*, 3: 248–253.

WRIGHT, H. L. 1940. Sea-salt Nuclei. *Quart. Jour. Roy. Meteor. Soc.*, 66: 3–12.

YOSHIMURA, S. 1936. The Effect of Salt-breeze on the Chemical Composition of Fresh Water Lakes near the Sea. *Arch. Hydrobiol.*, 30: 345–351.

ZOBELL, C. E. 1934. Microbiological Activities at Low Temperatures with Particular Reference to Marine Bacteria. *Quart. Rev. Biol.*, 9: 460–466.

————. 1941a. Studies on Marine Bacteria. I. The Cultural Requirements of Heterotrophic Aerobes. *Jour. Mar. Res.*, 4: 42–75.

————. 1941b. Occurrence of Coliform Bacteria in Oceanic Waters. *Jour. Bact.*, 42: 284.

ZOBELL, C. E. and CONN, JEAN E. 1940. Studies on the Thermal Sensitivity of Marine Bacteria. *Jour. Bact.*, 40: 223–238.

ZOBELL, C. E. and FELTHAM, CATHARINE B. 1934. Preliminary Studies on the Distribution and Characteristics of Marine Bacteria. *Bull. Scripps Inst. Oceanogr., tech. ser.*, 3: 279–295.

ZOBELL, C. E. and MATHEWS, HELEN M. 1936. A Qualitative Study of the Bacterial Flora of Sea and Land Breezes. *Proc. Nat. Acad. Sci.*, 22: 567–572.

ZOBELL, C. E. and MCEWEN, G. F. 1935. The Lethal Action of Sunlight upon Bacteria in Sea Water. *Biol. Bull.*, 68: 93–106.

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PHOSPHORITE DEPOSITS ON THE SEA FLOOR OFF SOUTHERN CALIFORNIA

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ABSTRACT

Dredging operations on banks, on escarpments, and on walls of submarine canyons off southern California have shown that nodular phosphorite is the most abundant type of rock in these nondepositional environments. Approximately one-third of all the rock recovered is phosphorite. Petrographic and megascopic examination reveals that the nodules are largely formed by direct precipitation, but that they enclose some replaced material. Examination also shows that the phosphorite was probably deposited essentially *in situ*. Miocene Foraminifera have been identified in the nodules from many of the stations, but Recent or Pleistocene faunas have also been found in some of the phosphorite. Whereas the enclosed Miocene fauna suggests a Miocene age for most of the nodules, significant nonpaleontological data indicate that this fauna has probably been reworked into more recent deposits before enclosure in the nodules.

INTRODUCTION

During the series of geological cruises of the E. W. Scripps in 1938, rock was dredged from a large number of stations off the southern California coast and from a few stations farther north. This rock will be described in a future paper by Emery and Shepard, but it was thought that special treatment should be given to the phosphorite because of its surprising abundance and because of the special problems which it raised. Phosphorite was recovered in greater abundance and was found in more localities than any other type of rock. Its abundance on the submarine surfaces in contrast to its scarcity in outcrops over any area of the same size on land, and the possibility that the present environment is

that in which the nodules were formed gave special interest to the investigation.

The large nodules were collected in bucket dredges (Shepard and Emery, 1940, Pl. 5, fig. 2). Many of the smaller ones were found in snapper samples and in cores of unconsolidated sediment.

Dietz made the principal laboratory study of the phosphorite samples but was assisted by Emery especially in the chemical investigation. Shepard conducted the marine investigation of the deposits. All of the writers have shared in the discussion of the origin of the material.

ACKNOWLEDGMENTS

The opportunity to collect samples from the sea floor was provided by a grant from the Penrose Bequest of the Geological Society of America. This grant made possible the use of the auxiliary schooner E. W. Scripps. The active co-operation of the Scripps Institution of Oceanography in field and laboratory has been important. Roger Revelle collected some of the samples and also assisted in a study of the solubility of calcium phosphate in sea water. The chemical data were checked by W. W. Rubey, W. L. Hill, K. D. Jacob, and W. F. Rodebush. For the age determination of Foraminifera of the phosphorite, the writers are greatly obligated to M. L. Natland of the Richfield Oil Company of California. Some Foraminifera were also identified by J. A. Cushman and S. G. Wissler. Critical readings of the manuscript were made by Eliot Blackwelder, M. I. Goldman, W. L. Hill, K. D. Jacob, G. R. Mansfield, W. W. Rubey, R. P. Sharp, H. R. Wanless, and R. C. Wells. Six chemical analyses of the phosphorite nodules were made by the U. S. Geological Survey. Laboratory aid was given by personnel of the Works Progress Administration, Official Project No. 665-07-3-141. The writers wish to express appreciation to these organizations and individuals.

DESCRIPTION OF THE PHOSPHORITE

GENERAL

About 600 kilograms of nodular phosphorite was dredged from 48 different stations, most of which are located off southern California (Pl. 3). The phosphorite ranges from oölites to large nodules (Pl. 1, fig. 4) measuring up to a maximum of 60 by 50 by 20 centimeters. Average diameter of the nodules is about 5 centimeters. Weights of the four largest nodules range from 35 to 73 kilograms.

EXTERNAL CHARACTER

The phosphorite varies in shape from thin flat slabs to nodular masses, but those from any particular dredge haul usually have a group resem-

blance. Smoothly rounded protuberances and cavities are present on some nodules (Pl. 1, fig. 1).

In general, the nodules are hard and dense and have a smooth glazed surface and a fresh appearance. Their surface consists of a thin discolored layer of phosphorite or of manganese oxide which obscures the internal character. Freshly broken surfaces are usually light to dark brown, but black surfaces are also found. At the four stations in the Santa Monica Bay area all of the phosphorite is black, both internally and externally.

In addition to occurring in nodular form, the phosphorite often coats hard compact rock and thoroughly permeates more porous varieties. Also, at several stations large pieces of conglomerate or breccia were dredged in which the phosphorite cements fragments of darker phosphorite and other types of rock (Pl. 1, fig. 2).

Many marine organisms including corals, bryozoans, sponges, serpulid worms, and brachiopods are found attached to the nodules. Some of the phosphorite is also deeply drilled by boring animals. In many specimens these borings and other cavities have become partially or completely filled with limy and phosphatic debris (Pl. 2, fig. 4). The presence of attached organisms growing on the top of a nodule indicates the position in which the nodule rested on the bottom. The upper surface is also sometimes identified by a thin black coating of manganese oxide which develops only on the exposed side, and indicates an interruption in phosphorite deposition. Such a coating is commonly present on other rocks dredged from the offshore steep slopes and banks.

INTERNAL CHARACTER

Phosphate minerals.—Petrographic examination of thin sections supplemented by chemical analyses show that collophane, composed of isotropic carbonate fluorapatite, is the principal mineral form in which the phosphate is present. Associated with the collophane is an anisotropic mineral which is probably francolite,[1] a carbonate fluorapatite. This mineral is present as a replacement of organic remains and other limy debris that was originally entrapped in the nodules. Francolite also exists as fibrous concentric rings in and around oölites, and as small grains disseminated throughout the collophane. Apparently it forms largely by the gradual crystallization of collophane and by the migration of some of the calcium phosphate to replace calcium carbonate.

[1] Although collophane and francolite are probably similar chemically, they differ optically, in type of crystallinity, and in other physical properties and, hence, were regarded as different minerals. However, the term collophane is a name for the microcrystalline equivalents of minerals of the apatite group including francolite. In most respects the difference between collophane and francolite is analogous to that between chalcedony and quartz.

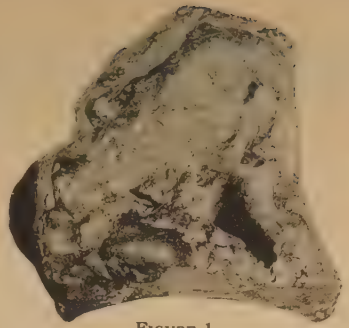


FIGURE 1.

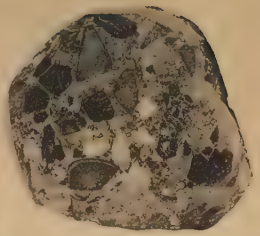


FIGURE 2.

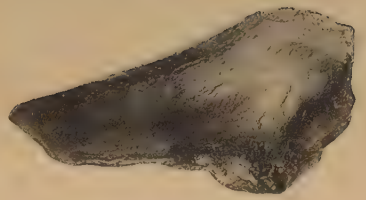


FIGURE 3.

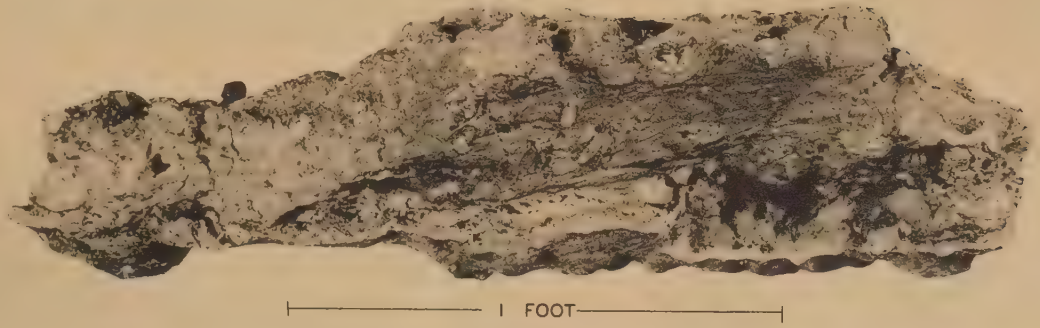


FIGURE 4.

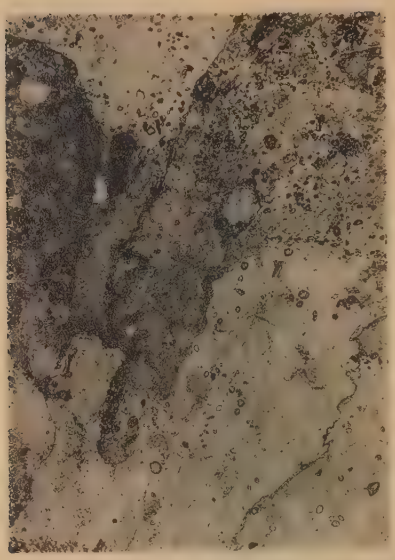
FIGURE 1. NODULE FROM STATION 70
Showing the typical external appearance. Note the glazed surface and irregularly rounded shape. X ½

FIGURE 2. BRECCIA FROM STATION 63 CEMENTED WITH COLLOPHANE.
The fragments in the breccia are mainly phosphorite although some andesite and chlorite schist are also present. X ½

FIGURE 3. PHOSPHATIZED SEA LION BONE FROM STATION 48. X ½.

FIGURE 4. LAYERING ON FRESHLY BROKEN SURFACE OF NODULE FROM STATION 70. X ¼.

PHOSPHORITE NODULES AND PHOSPHATIZED BONE



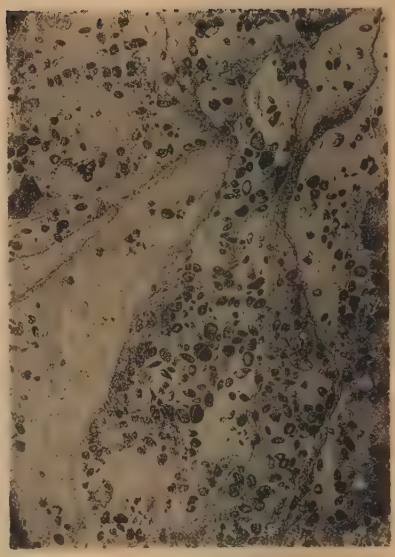


FIGURE 1 AND 2. PHOTOMICROGRAPHS OF NODULES FROM STATION 160
Polished sections with scattered oölites and mineral grains. Note the irregular layers which indicate many discontinuous periods of accretion. X 2½.

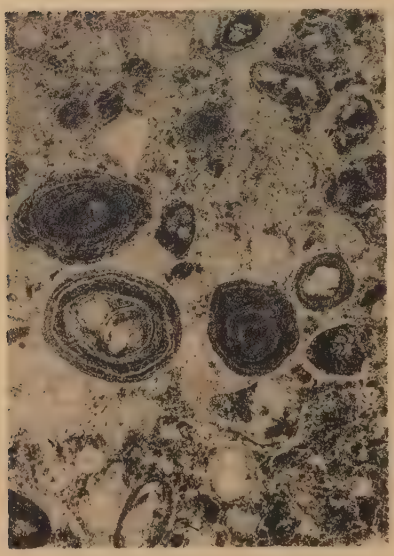


FIGURE 3. PHOTOMICROGRAPH OF A THIN SECTION OF NODULE FROM STATION 16
Under transmitted light shows typical texture. Note the concentric structure of the oölites. The nuclei consist of foraminiferal tests or mineral grains. X 28.

FIGURE 4. PHOTOMICROGRAPH OF A POLISHED SECTION OF A NODULE FROM STATION 162
Arrows point to animal borings which have been filled with sediment and subsequently phosphatized. X 3½.

INTERNAL CHARACTERISTICS OF PHOSPHORITE

Structure.—The structures of the nodules vary considerably in different samples and even within any particular haul. At a few stations there are pieces of homogeneous phosphorite composed entirely of massive collophane and francolite and having no enclosed material. However, the large majority of the nodules are layered and many are conglomeratic. The layers are irregular and nonconcentric (Pl. 2, figs. 1, 2), and vary in thickness from a few millimeters to a few centimeters. They are separated by a thin film of collophane of a darker shade of brown or less frequently by a thin coating of manganese oxide. Besides being layered, nearly all of the phosphorite is somewhat oölitic. However, oölites are much less abundant than in phosphorite samples from the Permian Phosphoria formation in which oölites form the mass of the rock.

Enclosed material.—A few phosphatic oölites are enclosed within the layers of phosphorite in most of the nodules (Pl. 2, figs. 1-3). The oölites are composed of concentric layers of collophane with an occasional layer of columnar francolite. Some of them have a definite nucleus (Pl. 2, fig. 3), which consists of a foraminiferal test, glauconite, or a clastic mineral grain. Usually, however, no nucleus can be discerned in thin section probably because the section failed to pass through the center of the oölite. In other instances, this absence is probably due to the complete absorption of a foraminiferal test nucleus. It is possible to find all stages in this absorption, from oölites having clearly defined tests of Foraminifera as nuclei to oölites in which only the faintest suggestion of the test remains.

Foraminiferal tests are also directly cemented into the nodules. Although a few tests are composed of unaltered calcium carbonate, most of them are more or less completely replaced by calcium phosphate usually in the form of francolite. In some nodules Foraminifera are extremely abundant and make up an appreciable part of the rock.

Although shells other than foraminiferal tests are not common within the phosphorite, fragments of corals, bryozoans, and other animal remains are found. At a number of stations, phosphatized bone fragments (Pl. 1, fig. 3) are present either separate or cemented into the nodules. These consist of bones of fish and mammals, the latter including a portion of a sea lion humerus and a whale's ear bone. At one station a phosphatized part of a turtle plastron was dredged. A number of other unidentified structures may be of organic origin.

Grains of clastic minerals are extremely abundant in some nodules but are absent in others. Some grains are rounded although more are angular. They consist mainly of feldspars, quartz, mica, and pyroxene.

Glauconite is abundant in nodules from most of the stations and occurs as rounded grains, as fillings of foraminiferal tests, as irregular masses, and as finely disseminated particles.

Insoluble residues.—To obtain insoluble residues, samples from many of the nodules were digested in hydrochloric or nitric acid. In typical samples this residue constitutes from 6 to 30 per cent, and averages 17 per cent.

The fine fraction of the residue consists dominantly of amorphous silica and carbonaceous material. Glauconite is extremely common in the coarse fraction of most samples, and in many cases predominates over all other types of material. As in the sediment associated with the nodules, much of the glauconite is coated with manganese oxide. In a few samples internal casts of foraminiferal tests are composed of glauconite or amorphous silica. Many of these casts preserve even the fine details of the tests and, consequently, were extremely helpful in studying the Foraminifera.

Also present in the coarse fraction of the insoluble residue are mineral grains, carbonaceous particles, and siliceous animal remains. The siliceous remains consist dominantly of sponge spicules, but include some diatoms and Radiolaria. A few samples of dark-colored phosphorite contain hollow oval carbonaceous casts and small (about 1 mm. in diameter) round oval carbonaceous pellets which may be coprolites.

ASSOCIATED SEDIMENTS

Examination of a large number of samples has shown that the sediments of the continental borderland [2] off southern California (Pl. 3) can be classified into four types: (1) shelf sediments consisting of sand and silt with abundant mica; (2) near-shore basin sediments consisting of micaceous silty clays and occasional sandy lenses; (3) offshore basin sediments consisting of fine clays containing abundant tests of pelagic Foraminifera; and (4) offshore bank sediments consisting of sand-size material largely nonclastic and composed of glauconite, foraminiferal tests, shells, and some detrital mineral grains, and rock fragments. This last type is found also on some steep slopes and in a few localities on the shelf and in submarine canyons.

The glauconitic sediment (type 4) distributed in patches over rocky bottom, indicates that these are essentially areas of nondeposition. Al-

[2] Continental borderland is a term applied to the rugged submarine topography of great relief lying between a coast and the continental slope which leads to the oceanic abyss. The term shelf is more properly restricted to the relatively flat zone of sea floor, mostly less than 400 feet deep, which rims the land.

though the sand is variable, a typical sample proved to be 60 per cent glauconite, 25 per cent foraminiferal tests and other animal remains, 10 per cent grains and oölites of phosphorite, and 5 per cent clastic minerals. Glauconite in the sands associated with the phosphorite has

TABLE 1.—*Chemical analyses*

	Station					
	69	106	127	158	162	183
CaO	47.35	45.43	45.52	46.58	37.19	47.41
R_2O_3	0.43	0.30	2.03	0.70	3.93	1.40
P_2O_5	29.56	29.19	28.96	29.09	22.43	29.66
CO_2	3.91	4.01	4.30	4.54	4.63	4.87
F	3.31	3.12	3.07	3.15	2.47	3.36
Org.	0.10	1.90	2.25	0.44	0.35	1.50
Insol in HCl	2.59	3.57	4.45	3.57	20.99	2.12
Total	87.25	87.52	90.58	88.07	91.99	90.32
F/P_2O_5	.1120	.1069	.1060	.1082	.1099	.1132
CO_2/P_2O_5	.1323	.1373	.1486	.1560	.2060	.1641

Analyses (excepting CO_2 and CaO) by U. S. Geological Survey.
CO_2 and CaO analyses by McVicker, Illinois State Geological Survey.

a dark green to black surface, the latter due to a coating of manganese oxide. Many of the grains are oval pellets or casts of foraminiferal tests. The composition of these sediments corresponds closely to material enclosed in the phosphorite nodules. Furthermore, phosphorite is almost invariably found associated with this type of sediment.

The association of phosphorite nodules with such deposits was also found by the Challenger expedition (Murray and Renard, 1891, p. 395-397). Furthermore, geologists have noted the common association of phosphorite nodules and glauconite in nondepositional horizons in sedimentary rock (Goldman, 1922).

CHEMICAL AND X-RAY INVESTIGATIONS OF THE PHOSPHORITE

CHEMICAL ANALYSES

Chemical analyses of nodules from six different stations were made by the U. S. Geological Survey. These analyses (Table 1) are surpris-

ingly uniform with the exception of sample 162 which differs mainly in the large amount of material insoluble in acid. The difference between the total percentage of the analyses and 100 per cent is probably largely MgO and soluble SiO_2 although small amounts of other substances must also be present.

The high fluorine content is of especial significance because it shows that the phosphate minerals are fluorapatites rather than hydroxyapatites. The amount of fluorine is slightly in excess of that required to completely fill with fluorine all the (OH, F) positions in the general apatite formula (Hendricks, *et al.*, 1931). This excess of fluorine is probably due to the replacement of some of the O positions by F rather than to the presence of a distinct fluorine mineral such as fluorite. Although fluorite has been detected in microscopic study of some phosphorites, for example the Phosphoria, fluorite was not found in the phosphorite from the sea floor off California.

In order to determine if the basin areas are the sites of unusually rapid deposition of phosphate, a series of phosphate analyses of muds from these environments was made by J. P. Cunningham. Samples were selected from the top and from the bottom of cores taken in basins at various depths and at different distances from shore. Phosphate varies from 0.3 to 0.5 per cent and shows no correlation with depth or distance from shore. The phosphate content is of the same order of magnitude as in the Carnegie samples which were dredged from the North and South Pacific (R. Revelle, personal communication), suggesting that only normal amounts of phosphate are found in the basin sediments.

X-RAY DATA

Although collophane is isotropic and, therefore has been considered amorphous, x-ray diffraction patterns show that the mineral is submicrocrystalline (Hendricks, *et al.*, 1931). X-ray studies by Dietz show that the phosphorite from off California, like other phosphorites, gives the typical apatite powder pattern. This apatitelike pattern is common to francolite and collophane.

In spite of the fact that calcium oxide (Table 1) is present in the phosphorite from the California sea floor in fairly large quantities, calcite has not been detected by x-ray technique. Consequently, the calcium must be a component of the phosphate minerals.

Phosphorite from off California gives a pattern identical with that of a sample from the Phosphoria formation (Permian) at Garrison, Montana, except that the lines of the latter are not as broad. This indicates that the material from the Phosphoria is composed of crystals having

a larger particle size. If the samples are representative of the deposits from which they were obtained, this comparison may illustrate the well-known tendency for amorphous and submicrocrystalline substances to become more coarsely crystalline after a lapse of time.

PALEONTOLOGICAL DATA

VERTEBRATE REMAINS

A number of phosphatized vertebrate remains such as teeth, vertebrae, the ear bone of a whale, and other bones, were enclosed or associated with phosphorite. Bones of mammals, probably marine, and of fish were well represented. A phosphatized sea lion bone (Pl. 1, fig. 3) was dredged along with about 200 phosphorite nodules at Station 48. This bone was identified by Dr. Remington Kellog as the lower end of the shaft of the right humerus of a sea lion which closely resembles though differs from the Recent *Eumetopias stelleri*. The geologic range of this species extends into part of the Pleistocene. According to Kellog the specimen differs from the bones of a related Miocene (Temblor) sea lion although it may possibly fall within the range of variation of this species. Other bones were too fragmentary for age determination.

FORAMINIFERA

General.—All significant paleontological data regarding age were obtained from a study of Foraminifera. The identification of these Foraminifera has been almost entirely the work of M. L. Natland, although S. G. Wissler and J. A. Cushman have also made certain identifications.

Fauna of the associated sediment.—The sediment associated with the nodules contains abundant Foraminifera and in many cases it is predominantly composed of these organisms. Not only Recent Foraminifera are present but also many reworked fossil types, predominantly Miocene, which are often worn, fragmentary, and partially dissolved. At Station 70, almost half of the Foraminifera brushed off the surface of the nodules were Miocene forms. In contrast, mud in cores from the basins in this area rarely contain reworked fossils.

According to Natland, the Recent faunas present in the associated sediments and the Recent (or Pleistocene) faunas detected in a few nodules are, in a general way, indicative of the depth at which they are now found.

Fauna of the associated nonphosphatic rocks.—A study of the rocks dredged from the ocean floor off southern California has disclosed the presence of abundant sedimentary rocks containing Foraminifera. These

organic remains show that the rocks are dominantly Miocene. A specimen of very slightly consolidated mudstone from Station 273 contained a mixed fauna with forms diagnostic of Saucesian, Luisian, Mohnian, Delmontian, and Repetto age (lower Miocene to lower Pliocene, Table 2).

TABLE 2.—*Stratigraphic sequence of faunal units*
(Adapted from Kleinpell, 1938, Fig. 14)

Periods		So. Calif. Faunal Units
Recent and Pleistocene		Recent, Pleistocene, and uppermost Pliocene
Pliocene		Pico
		Repetto
Miocene	Upper	Delmontian
		Mohnian
	Middle	Luisian
		Relizian
	Lower	Saucesian
		Zemorrian

Fauna of the phosphorite.—Many of the phosphorite nodules contain poorly preserved foraminiferal tests most of which are completely replaced by francolite. The rock usually splits through rather than around these tests, making it necessary to identify the Foraminifera from cross sections. However, insoluble residues of some of the nodules yielded well-preserved internal casts composed of glauconite and silica. Quaternary Foraminifera which are unaltered and in an excellent state of preservation were found in some of the nodules.

Diagnostic Foraminifera were found in the nodules from 15 stations. These stations include nearly all those at which a large number of nodules were dredged. The foraminiferal data are briefly summarized as follows:

STATION 16

Foraminifera include *Anomalina salinasensis* Kleinpell, and *Siphogenerina branneri* Bagg. Relizian or L. Luisian (m. Miocene).

STATION 45

Among the Foraminifera present are: *Baggina robusta* Kleinpell, and *Valvulineria californica* Cushman. Luisian (u. m. Miocene).

STATION 48

Foraminifera include *Bolivina californica* Cushman, *Bulimina uvigerinaformis* Cushman and Kleinpell, and *Pulvinulinella gyroidinaformis* Cushman and Goudkoff. Lower Mohnian (l. u. Miocene).

STATION 63

Cassidulina fauna of the Lomita Quarry type (Galloway and Wissler, 1927) is present in the matrix which also encloses numerous nodules which on the basis of lithology are probably Miocene. A characteristic form is *Cassidulina subglobosa* var. *quadrata* Cushman and Hughes. (u. Pliocene to Recent)

STATION 64

The phosphorite in this sample fills pholad borings in an andesite cobble. A *Cassidulina* fauna of the Lomita Quarry type includes *Cassidulina subglobosa* var. *quadrata* Cushman and Hughes, and *Cassidulina limbata* Cushman and Hughes. (u. Pliocene to Recent)

STATION 69

A boulder-size nodule from this station consists of five layers. The bottom layer is conglomeratic and contains in the matrix a rich and well-preserved *Cassidulina* fauna of the Lomita Quarry type. Foraminifera present include *Cassidulina californica* Cushman and Hughes, *C. reflexa* Galloway and Wissler, *C. tortuosa* Cushman and Hughes, *C. limbata* Cushman and Hughes, and *C. subglobosa* var. *quadrata* Cushman and Hughes. This fauna is uppermost Pliocene to Recent. Small nodules enclosed in the matrix contain Luisian or Relizian (m. Miocene) Foraminifera: *Baggina* cf. *robusta* Kleinpell and a Miocene type of *Bolivina*. The upper four layers of this nodule are dark brown to black and contain a few poorly preserved Foraminifera of upper or middle Miocene (*Baggina robusta* Kleinpell and a Miocene *Valvulineria*). Inside this nodule are a number of pockets which have no exterior connection. Foraminifera in unconsolidated sediment in these pockets are Pleistocene or Recent. This fauna, however, differs from the Recent fauna scraped from the surface of the nodule.

STATION 70

Nodules from this station contain a middle and upper Miocene fauna including *Baggina californica* Cushman, *Bolivina imbricata* Cushman, *Bolivina sinuata* Galloway and Wissler, *Cassidulina crassa* d'Orbigny, *Planulina ornata* d'Orbigny, *Pullenia miocenica* Kleinpell, and *Uvigerinella* cf. *obesa* Cushman. Although the fauna in the sediment clinging to the surface of the nodules is predominantly Recent, an abundance

of reworked Miocene Foraminifera were found including *Siphogenerina collomi* Cushman, *Cassidulina monicana* Cushman and Kleinpell, and *Uvigerina hootsi* Rankin.

STATION 106

The largest nodule contains Miocene Foraminifera such as *Nodogenerina avana* Cushman and Laiming (l. m. Miocene).

STATION 156

A nodule from this station contains a Mohnian fauna (l. u. Miocene).

STATION 158

Nodules have a mixed fauna including the Miocene form *Uvigerina hootsi* Rankin and a *Cassidulina* fauna of the Lomita Quarry type which is uppermost Pliocene to Recent.

STATION 159

Nodules contain *Valvulineria californica* Cushman, *Bolivina advena* Cushman, *Nonion costiferum* (Cushman), and others (l. m. Miocene).

STATION 162

The phosphorite is conglomeratic with a matrix having a well-preserved *Cassidulina* fauna of the Lomita Quarry type including *Cassidulina tortuosa* Cushman and Hughes, *C. subglobosa* var. *quadrata* Cushman, *C. limbata* Cushman and Hughes, *C. californica* Cushman and Hughes, *Bulimina ovula* d'Orbigny, and *Planulina ornata* d'Orbigny (uppermost Pliocene to Recent). An enclosed nodule contains a Miocene *Siphogenerina.*

STATION 183

Foraminifera present include *Baggina californica* Cushman, *B. robusta* Kleinpell, *Bulimina uvigerinaformis* Cushman and Kleinpell, *Bulimina ovula* d'Orbigny, *Cassidulina monicana* Cushman and Kleinpell, *Cassidulina modeloensis* Rankin, *Eponides healdi* Stewart and Stewart, *Nodogenerina advena* Cushman and Laiming, *Planulina ornata* d'Orbigny, *Virgulina californiensis* Cushman, *Uvigerina* cf. *senticosa* Cushman. Predominantly Mohnian (l. u. Miocene).

STATION 303

Foraminifera present include *Dentalina obliqua* (Linné) Woodring, Bramlette, and Kleinpell, *Nodogenerina advena* Cushman and Laiming, *Valvulineria* sp. Miocene or Pliocene type, *Bolivina* sp. Miocene or Pliocene type. Luisian or Relizian (m. Miocene).

STATION 9604

Bolivina girardensis Rankin is present. Relizian or Luisian (m. Miocene).

STATION 9610

A single *Siphogenerina* was found. (m. Miocene (?)).

Apparently the bulk of the material contains middle Miocene Foraminifera, but nodules from a few stations have upper Miocene Foraminifera. A Quaternary fauna which extends into uppermost Pliocene is present in some separate pieces of phosphorite and in layers superimposed upon a few nodules containing Miocene Foraminifera. The quantity of material containing this Quaternary fauna is relatively small as compared to the quantity containing the Miocene fauna. In one instance, a questionable Repetto (Pliocene) fauna was recognized.

Although both Recent and Miocene Foraminifera have been identified in some nodules, these faunas are confined to separate layers except in a very few specimens. While the identification is not certain, a number of nodules from Station 158 contain faunas composed of mixed Miocene, Pliocene, and Recent forms. A few other nodules contain a mixed fauna of Foraminifera characteristic of different faunal units of the Miocene.

The nodules containing Quaternary Foraminifera differ sufficiently in physical appearance from those containing Miocene tests to permit distinction on lithology alone. In general, they are characterized as follows: (1) They are light brown on fresh fracture; (2) they contain well-preserved Foraminifera which are usually unaltered or only partially replaced by francolite, and (3) they are conglomeratic, enclosing dark-brown to black pebbles of phosphorite having Miocene Foraminifera. Phosphorite containing Quaternary Foraminifera occurs as separate nodules and as layers superimposed upon some of the nodules having a Miocene fauna.

In contrast, most of the nodules having Miocene Foraminifera are characterized by the following: (1) They are dark brown to black, (2) they contain foraminiferal molds or poorly preserved tests completely replaced by francolite, and (3) they contain many layers, some of which are separated by manganese oxide.

Inasmuch as some nodules having a Miocene fauna are superimposed by a layer of different lithologic appearance which encloses a Quaternary fauna, it is apparent that there are at least two distinctly different times of phosphate deposition. Although phosphorite which contains the Quaternary Foraminifera must be of Quaternary age, those nodules having Miocene fauna were not necessarily deposited during the Miocene. Miocene Foraminifera may have been reworked into younger deposits

or phosphatic solutions may have infiltrated unconsolidated Miocene deposits. These considerations will be more fully discussed later in the paper.

DISTRIBUTION OF PHOSPHORITE

WORLD DISTRIBUTION

Continental distribution.—Marine deposits of nodular phosphorite, and bedded phosphorite into which they grade, are fairly common in the geological column, and range from Cambrian to Pleistocene. It has even been suggested (Dawson, 1876) that some of the rich apatite beds that are intimately associated with the pre-Cambrian Grenville marbles and gneisses near Ottawa, Canada, are highly metamorphosed representatives of phosphatic marine sediments. Among the best known phosphorite deposits are those of the Devonian of Tennessee, the Mississippian and Permian of the Rocky Mountains, the Cretaceous at Taplow in England, the Tertiary of Tunis and Algiers, and the Miocene and Pliocene of Florida. Some of these deposits extend over an area of thousands of square miles.

General oceanic distribution.—Since oceanographic expeditions have dredged phosphorite nodules in a considerable number of rocky portions of the ocean floor, it seems likely that phosphorite is relatively common on the ocean floor. The CHALLENGER Expedition (Murray and Renard, 1891) obtained the nodules on Agulhas Bank, south of the Cape of Good Hope; off the east coast of Japan; off the coast of Spain; off the east coast of Australia; off the coast of Chile; between the Falkland Islands; and off the mouth of the Plata River. More nodules were later dredged from Agulhas Bank by the German ships, GAZELLE and VALDIVA (cited by Collet, 1907), and by the Cape Government (Cayeux, 1934). The American ship BLAKE (Agassiz, 1888) dredged similar concretions off the east coast of the United States and in the Straits of Florida and later the ALBATROSS (Murray and Lee, 1909) obtained some nodules from the north Pacific. Published descriptions show that nodules from each of these localities agree closely in megascopic and microscopic character with those dredged off California. (*See* especially Cayeux, 1934.)

SUBMARINE DISTRIBUTION OFF SOUTHERN CALIFORNIA

General.—Plate 3 shows the location of stations where phosphorite was obtained off the southern California coast. Of 131 samples with rock, 43 contained phosphorite of a diameter greater than 5 millimeters. At some other stations partially phosphatized rock was found, and at others sand containing grains of phosphorite.

A near-shore zone, which is limited by the outer margin of the shelf, has a total of 49 rock hauls but only 8 of these contain phosphorite. It is notable that all but 2 of the latter were located off land areas where Miocene outcrops are dominant. The offshore zone beyond the shelf has a total of 82 rock hauls, of which 35 have phosphorite, a percentage of 43 compared with 16 per cent for the inshore zone. By subdividing the offshore zone into northern, central, and southern portions (Pl. 3), it was found that the southern portion yielded 6 rock hauls, none of which had phosphorite, whereas the central portion has phosphorite in 32 out of 65 rock hauls; and the northern portion in only 3 out of 11 hauls. These figures show that in the central portion 49 per cent of the hauls contain phosphorite compared to 33 per cent for the entire submarine area off southern California. Numerous Miocene outcrops have been found on the islands and the sea floor of this central portion.

Physiographic distribution.—Examination of Plate 3 shows that phosphorite is not only widespread, but also occurs in a considerable variety of topographic environments. It was obtained on the top and sides of banks, on steep escarpments which appear to be fault scarps, on walls of submarine canyons, and on the continental shelf. All of the localities where phosphorite is found are essentially nondepositional environments.

Dredgings brought up rock from nine canyons off the southern California coast. Phosphorite was obtained from the walls of three,—those off Redondo, Santa Monica, and Point Dume. Rock samples are available from 20 banks, 10 of which have yielded phosphorite. Since most of the banks which lack phosphorite have had only one dredging some of them may show the presence of phosphorite when more dredgings are available. San Juan Seamount, while not classed as a bank since it rises as a great mountain from the deep ocean floor, did not yield phosphorite. This seamount is thought to be a volcanic mass (Shepard and Emery, 1941, p. 28, 29).

The supposed fault scarps have not been very completely explored for phosphorite. However, the two counterposed scarps southeast of San Clemente Island, which form a V-shaped valley where they overlap (Shepard and Emery, 1941, Fig. 11) were dredged extensively and yielded phosphorite in 7 out of 17 hauls. Dredgings also brought up this material from other similar escarpments.

Rock has been dredged from eight localities on shelves including those around islands. Only the shelf off Santa Monica and that off San Diego yielded phosphorite. No phosphorite was found in the rocks from the shelf off Mission Beach (near San Diego), off Carlsbad, off Long Beach,

off San Nicolas Island, and on the small knob which rises above the shelf near the western end of the Santa Monica Mountains.

Depth and relation to shore.—There is little evidence of a lower depth limit for the material, nor any suggestion of a maximum concentration at any depth. The deepest dredging, which was taken at 10,320 feet on the continental slope, did not yield phosphorite, but it was found in a nearby sample at about 8400 feet. The CHALLENGER Expedition dredged some nodules from a depth of 11,400 feet at the base of the continental slope off the Cape of Good Hope. The shoalest sample of phosphorite was recovered from 240 feet on the Santa Monica Shelf. This haul (Station 303) contained an abundance of phosphorite. Rock dredged from 10 shoaler localities included no phosphorite. This does not prove that phosphorite is absent in shoal water, but it suggests this possibility. This is contrary to the hypothesis of Anderson (1895) that phosphorite predominates in littoral deposits.

Little phosphorite has been found close to shore, either on the continental shelf or on the insular shelves. On the other hand, almost half of the hauls from the offshore area contain phosphorite. The material is abundant in hauls made in the outer portions of the 150-mile wide continental borderland, but was not found in the few samples beyond the continental slope. Inasmuch as phosphorite has never been reported from the abyssal ocean basins, it may be that phosphorite, like glauconite, is formed only relatively close to shore.

Distribution in adjacent regions.—Although the data are few, phosphorite appears to occur in less abundance to the north and south of the southern California continental borderland. Twenty-three rock samples were collected by the E. W. SCRIPPS and the BLUEFIN to the north of this region, particularly in Monterey Bay. Phosphorite is present in 3 of these samples. In addition examination was made of samples collected by the Coast and Geodetic Survey along the west coast of North America. Over 100 of these sediment samples collected between Lats. 36° and 60° were studied. Although many of these contain rock fragments and are highly glauconitic, no phosphorite was found. In an expedition to the Gulf of California in 1940, 30 samples with rock were recovered of which 6 contained phosphorite.

OCCURRENCE ON LAND IN SOUTHERN CALIFORNIA

Although phosphorite is abundant on the sea floor, study of the literature, consultation with other geologists, and field work have shown that it is relatively uncommon on the mainland and apparently absent on the islands. Miocene and to a lesser extent Pliocene and Pleistocene rocks

on the mainland contain a small amount of phosphorite. Drilling in the Playa del Rey and the Torrence oil fields on the west end of the Los Angeles Basin encountered 150 feet of upper Miocene black shale (Hoots, *et al.*, 1935) which has a spotted and a nodular nature due to the erratic occurrence of tan and gray phosphorite nodules from 1 to 8 centimeters in diameter. Some of these are porous and a few have an indistinct oölitic structure. The nodules contain about 70 per cent tri-calcium phosphate and are associated with thin discontinuous tan-colored bands of the same composition; however, they are distinctly different in size, shape, and structure from those dredged offshore.

A dark-colored bituminous nodular shale 200 feet thick, also upper Miocene, is exposed half a mile south of Capitan Point, north of Ventura. In many ways this deposit is similar to that of the oil fields. Inasmuch as the phosphorite nodules occur along bedding planes and not along joints and since they are in a fine-grained deposit, they were apparently formed syngenetically and in place.

One of the most interesting deposits of phosphorite found on land in California is at the Lomita Quarry on the north side of the Palos Verdes Hills. Pieces of phosphorite as long as 25 centimeters occur in a glauconitic marl and also in association with a band of subangular to subround rocks at a slightly higher horizon. In this band the nodules predominate over other types of rock. Although the enclosing beds are lower Pleistocene (or uppermost Pliocene) (Galloway and Wissler, 1927), M. L. Natland and H. L. Driver (personal communications) have identified Miocene Foraminifera in the phosphorite. Consequently the nodules, or at least the Foraminifera, have been reworked out of Miocene formations. In contrast to the phosphorite from the Miocene nodular shale, the material from the Lomita Quarry resembles that dredged from the ocean floor in color and in the presence of oölites, banding, glauconite, and Foraminifera.

Another occurrence of phosphorite is in a near-by road cut a quarter of a mile south of the Lomita Sand and Gravel Company. These nodules are in a well-sorted basal gravel bed unconformably overlying tilted Miocene beds. About one third of the pebbles in one zone are phosphorite.

ORIGIN OF THE PHOSPHORITE

PREVIOUS VIEWS

Many writers have attempted to explain the origin of phosphorite and the physical-chemical conditions which caused the precipitation of phosphate. Blackwelder (1916) and Mansfield (1927, p. 365) suggested that this precipitation may take place in sea water low in oxygen. These

writers have pointed out that an oxygen deficiency exists in submarine basins where stagnant conditions are reflected in the high content of organic matter in the sediments. This stagnant water inhibits the growth of organisms and may allow phosphate to become sufficiently concentrated to produce chemical reactions that are not ordinarily operative. The ion thus becomes fixed in the form of nodules or continuous beds of phosphorite. Although this may be the correct explanation for many phosphorites and for phosphatic shales, it probably does not account for the phosphatic nodules off California since the latter appear to have formed in association with coarse-grained glauconitic sands on topographic highs. Furthermore, it has been shown (Trask, 1932, p. 120; Revelle and Shepard, 1939, p. 262) that the sediments from the topographic highs off California where the phosphorite is found are relatively low in organic matter. In addition, oceanographic data show that these areas are among the most oxidizing environments of the sea floor (Revelle, personal communication).

In order to account for the occurrence and distribution of the phosphorite nodules dredged by the CHALLENGER Expedition, Murray and Renard (1891, p. 396-399) postulated a catastrophic destruction of life resulting from the mixing of a cold water mass with a warm water mass as might occasionally occur along the border of a great ocean current. According to Murray and Renard, organic matter would be deposited in such abundance that special chemical conditions might operate so that phosphate would not be returned to the sea water, but rather would be precipitated as nodules. Later, Murray (1898, p. 113) cited a remarkable case in which tile-fish were killed by the hundreds of millions along the Atlantic Coast of the United States in 1883, presumably due to the shifting of oceanic currents. However, Blackwelder (1916, p. 290) has pointed out that dead fish float and Mansfield (1927), in attempting to apply this theory to the Phosphoria formation, states that the relative rarity of animal remains such as fish teeth is incompatible with catastrophic killing. The same objections apply to such an origin for the deposits off the California coast.

Pardee (1917) has suggested that the deposition of the Phosphoria formation was due to the same cool climatic conditions that produced the glaciers known to have existed during the Permian period. Carbon dioxide, supplied not only by the atmosphere but also by decaying organic matter on the sea bottom, is retained most abundantly by waters of low temperature. In such water calcareous remains would tend to be dissolved and the accumulation of lime deposits would be hindered; consequently, the phosphatic substances would accumulate in relatively pure

form. However, it is apparent that a glacial climate is not essential for the deposition of phosphorite because similar deposits range from Cambrian to Recent and widespread glacial conditions were infrequent during this interval. The possible Pleistocene age of the deposit off California might be thought to support this theory of Pardee, but most of the nodules were dredged from depths greater than 1000 feet, and some of them also contain deep-water Foraminifera indicating that the nodules were formed at depths similar to those in which they are now found. Sea water this far below the surface has a uniform temperature of a few degrees above zero centigrade; consequently, it would be possible for this water to become only slightly colder. Such a small change probably would not exert an important influence upon the chemical equilibrium of the sea water.

Mansfield (1940b) has suggested that fluorine may be responsible for the deposition of phosphorite. He pointed out that most phosphorites are composed of fluorapatite and were probably originally deposited as this material.

Since fluorine in the form of HF is a common constituent of volcanic gases, Mansfield maintains that times of intense volcanism are favorable to the deposition of phosphorite. The California phosphatic shales are Miocene,—a time of widespread volcanism in this region (Reed, 1933, p. 203).

CHEMISTRY OF PHOSPHATE IN THE OCEAN

General.—The mineral apatite, a minor primary constituent of practically all igneous rocks, is regarded as the principal original source of the sedimentary phosphates (Blackwelder, 1916, p. 286). The phosphorus liberated in the decomposition of rocks on land is ultimately carried in solution to the sea where plants and animals extract small quantities for use in their body structures. Phosphorus is an essential constituent of protoplasm and an important constituent of bones, teeth, and many shells. After the death of an organism, its remains are devoured by scavengers and decomposed by bacteria, with the result that the phosphorus is returned to the ocean water. From time to time, phosphorus escapes this apparently endless cycle and becomes fixed in the sedimentary deposits. An understanding of this fixation process requires a consideration of the chemistry of phosphate in sea water.

Solubility data for $Ca_3(PO_4)_2$.—It was felt that an investigation of the chemistry of phosphates in sea water might lead to a clearer understanding of the methods by which phosphorite and phosphatic shales are formed. So far as the writers could determine, no data are available in the literature regarding the solubility of phosphorite nor of its components, collophane and francolite. However, some experimentally deter-

mined constants for $Ca_3(PO_4)_2$ have been published by Sendroy and Hastings (1927). They found the following equation to hold in a salt solution at 38° C. where μ is the ionic strength of the solution.

$$PK'_{S.P.\ Ca_3(PO_4)_2} = 30.95 - \frac{17.40\mu^{\frac{1}{2}}}{1+1.14\mu^{\frac{1}{2}}} \qquad (1)$$

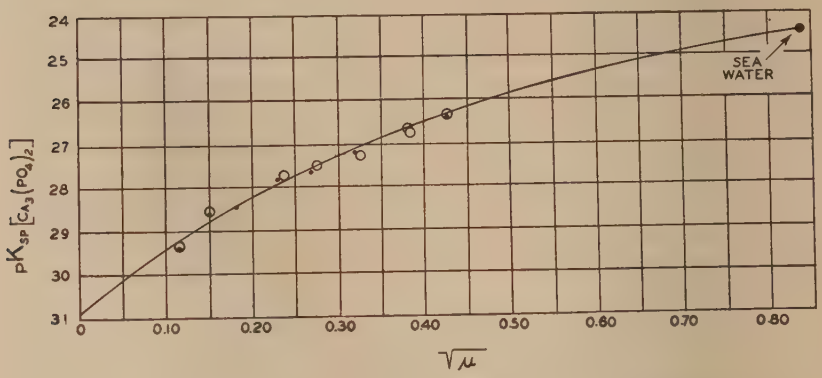


FIGURE 1.—*Relationship between ionic strength* (μ) *and solubility of* $Ca_3(PO_4)_2$ Showing extrapolation of Sendroy and Hastings data (circles, dots, and crosses) to sea water.

The ionic strength of sea water may be determined from the equations given by Lyman and Fleming (1940, p. 141), namely:

$$\mu \text{ for sea water} = 0.00147 + 0.03592\ (Cl\ ‰) + 0.000068\ (Cl\ ‰)^2 \qquad (2)$$

where (Cl‰) is the chlorinity in grams per kilogram. The chlorinity of the sea water averages about 19‰ but may be only 18‰ or 17‰ at relatively slight depths (Thompson and Robinson, 1932, p. 112). Accordingly substituting from (2) into (1) above,[3] when the chlorinity is approximately 19‰:

$$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2 = K'_{S.P.\ Ca_3(PO_4)_2} = 3.73\ (10)^{-25} \qquad (3)$$

Substituting these values in (3) and transposing:

$$[PO_4^{\equiv}] = \sqrt{\frac{3.73\ (10)^{-25}}{(0.010)^3}} = 0.61\ (10)^{-9} \text{ mols per liter} \qquad (4)$$

This value represents the amount of $[PO_4^{\equiv}]$, which theoretically can be present in sea water.

From oceanographic work, total phosphate is known to increase from about zero at the surface to over 200 milligrams per cubic meter at depths

[3] The ionic strength of sea water is greater than that of the salt solution used in the experiments of Sendroy and Hastings. However, the calculated sea water values for $\mu^{\frac{1}{2}}$ of 0.84; and for $K'_{S.P.\ Ca_3(PO_4)_2}$ of 3.73 $(10)^{-25}$ probably represents a safe extrapolation of their data (Fig. 1).

of several thousand meters in the open sea. Values of phosphate given in oceanographic reports are simply the sum of the PO_4 radicals, no attempt being made to determine the concentration of the dissociated ions. However, the dissociation constants of phosphoric acid used by Sendoy and Hastings (p. 786-790, 801) may possibly be extrapolated for sea water. For a solution in which $\mu = 0.71$, these constants are:

$$PK'_1 = 1.69$$
$$PK'_2 = 6.10$$
$$PK'_3 = 10.77$$

Thus, the corresponding values of K′:

$$\frac{(H^+) \times [H_2PO_4^-]}{[H_3PO_4]} = 2.04\ (10)^{-2} \qquad (5)$$

$$\frac{(H^+) \times [HPO_4^{=}]}{[H_2PO_4^-]} = 7.95\ (10)^{-7} \qquad (6)$$

$$\frac{(H^+) \times [PO_4^{\equiv}]}{[HPO_4^{=}]} = 1.70\ (10)^{-11} \qquad (7)$$

In sea water having a total phosphate content of 100 milligrams per cubic meter, or $1.05\ (10)^{-6}$ mols per liter:

$$[H_3PO_4] + [H_2PO_4^-] + [HPO_4^{=}] + [PO_4^{\equiv}] = 1.05\ (10)^{-6} \qquad (8)$$

Thus, if values of (H^+) are substituted in (5), (6), and (7) we have four equations with four unknowns, namely, (5), (6), (7), and (8) from which $[PO_4^{\equiv}]$ may be determined. From these equations and at a pH of 8, $[PO_4^{\equiv}]$ is $1.76\ (10)^{-9}$ mols/l. Dividing this value by the theoretical saturation concentration (4), we find that such a solution would be 289% saturated.

Sources of error.—In the derivation of the solubility of $Ca_3(PO_4)_2$ use was made of the solubility product determined at 38° C., and the best available ionic dissociation constants, some of which were also determined at 38° C. However, sea water always has a much lower temperature, and at depths of a few hundred meters in the open sea is less than 10° C.; accordingly these constants may be somewhat incorrect.

The solubility of $Ca_3(PO_4)_2$ is undoubtedly influenced to some extent by pressure (Gibson, 1938), and by temperature, but relatively little is known of the effect of these factors. Another possible source of error exists in the unknown effect of certain other ions which may make $Ca_3(PO_4)_2$ more or less insoluble. One such ion is F^-, which, according to Mansfield (1940b, p. 867) may be responsible for the deposition of phosphate in a relatively insoluble form. Adler, *et al.* (1938) point out that $Ca_3(PO_4)_2$ is an effective adsorbent of F in ground waters.

Further error results from the use of chemical data based on $Ca_3(PO_4)_2$, whereas phosphorite is a tri-calcium phosphate containing water, carbonate, and fluorine. In addition, the law of Debye and Hückel (which states that the activity of an ion in salt solution is dependent on the ionic strength of that solution) is correct only as a first approximation.

Application of the solubility data.—Although these calculations concerning the degree of saturation of sea water with tri-calcium phosphate can be only approximate, significant conclusions are suggested. For example, it appears probable that sea water deeper than a few hundred meters is essentially saturated with tri-calcium phosphate. If the ocean is saturated, it follows that an amount equal to all the phosphate annually carried to the sea by rivers must be deposited as phosphate minerals or as organic matter. Also such saturation may allow the existence of a colloidal phase of tri-calcium phosphate in equilibrium with the dissolved phase. It has been pointed out above that phosphorite is composed of colloidal particles, and this is at least suggestive that precipitation took place from a colloidal suspension.

Owing to the dependence of the phosphate saturation point on pH, and the wide variation in phosphate content in the sea, slight changes in physical-chemical or biological conditions may bring about supersaturation or undersaturation. Changes brought about by the activity of benthonic organisms may cause solution or precipitation of phosphorites. Thus the calcium phosphate balance in the sea is similar to that of calcium carbonate.

DEPOSITION ESSENTIALLY *IN SITU*

Nodules of phosphorite may form *in situ* or may have been transported to their present positions. The following considerations suggest that a large portion of the phosphorite off the California coast was formed essentially in place:

(1) The mammillary surfaces formed by growth layers on many nodules are present on only one side, and these same sides have attached organisms and coatings of manganese oxide. This association indicates that at least many of these particular nodules have grown in place and have not been subsequently disturbed.

(2) The very large size of many of the nodules and their lack of abrasion rounding suggest absence of transportation and even of disturbance by waves after the nodules were formed.

(3) The discovery of sediment within the nodules which closely resembles the surrounding sediment suggests that the nodules have formed in place. For the same reason Murray and Renard (1891, p. 397) came

to the conclusion that the nodules on the sea floor off the Cape of Good Hope were formed *in situ*.

(4) Attention has been called previously (Goldman, 1922) to nondepositional environments in the stratigraphic column as favorable for the accumulation of nodular phosphorite. The phosphorite is found in just such environments off southern California.

Additional considerations which oppose long distance transportation include: (1) There should be some remnant of the source beds on land, and (2) no method is known whereby the largest nodules could have been transported any appreciable distance from their source beds.

ORIGIN OF NODULES

Phosphorite nodules and closely related beds of marine phosphorite have been explained by direct deposition from solution or by replacement of other rocks. Murray and Renard (1891, p. 399) suggested that colloidal calcium phosphate supplied from decaying organisms is precipitated about some nucleus and then grows by attraction of like particles. These writers also pointed out that ammonium phosphate liberated by organisms may replace calcium carbonate. In support of this idea, they cited the experiment of Irvine and Anderson (1891, p. 52-54) in which the immersion of a porous variety of coral in a solution of ammonium phosphate for 6 months resulted in a replacement of 60 per cent of the calcium carbonate by calcium phosphate. Blackwelder (1916) has also suggested that ammonical solutions, derived from the decay of marine organisms, dissolve phosphatic material from bones, teeth, and shells. This phosphate may then locally replace carbonates and enrich the calcium phosphate in bones and phosphatic shells, although Blackwelder thought it would be precipitated directly to a greater extent, forming nodules and bedded phosphorite. Replacement of rock by phosphatic solutions has also been reported in localities where the rock was overlain by guano (Clark, 1924, p. 525).

The following considerations appear to indicate that the nodules off California are primary, that is, the result of direct precipitation:

(1) Practically all of the nodules are composed of irregular nonconcentric layers of brown collophane separated by a thin film of darker shade. These films have all the characteristics of the coatings found on the surface of the nodules, indicating that they are former surface coatings and showing that the nodules were formed by discontinuous accretion. The lapse of time between these periods of accretion is commonly shown by a coating of manganese oxide and by the presence of a band of glauconite grains above the dark collophane film.

(2) Tarr and Twenhofel (Twenhofel, 1932, p. 533-535) have shown that colloidal particles of silica because of their size probably lack the ability to penetrate dense limestone and consequently cannot replace them. Tarr's observations suggest that fossils in chert and elsewhere are never replaced by chert but rather by quartz. Similarly, the foraminiferal tests in phosphorite nodules are not composed of microcrystalline colloidal collophane but rather of francolite apparently formed by ionic replacement. The nodules, however, are largely composed of collophane, the colloidal particles of which could not have penetrated and replaced dense limestone. Some phosphorite was found coating massive limestone, but this coating is separated from the limestone by a sharp contact beneath which the limestone is essentially unaltered.

(3) The nodules have distinctive textural features unlike those which would be expected if a limestone or a related sedimentary rock had been replaced. In this connection, the enclosure within the nodule of bones, glauconite, coarse clastic mineral grains and rock fragments is significant because their abundance is greater than is usual in calcareous rocks.

Although the majority of the nodules are apparently of primary origin, most of them enclose a considerable amount of replaced material that was originally calcareous. Foraminiferal tests replaced by francolite are extremely common and form an important part of some of the nodules. In all thin sections, scattered anisotropic particles of francolite are present. Probably these are replacements of finely comminuted calcium carbonate, like that found within the associated sediments.

Much of the calcareous sediment associated with the nodules gives a strong chemical test for phosphate indicating that some phosphatization has taken place. A few pieces of homogeneous phosphorite, which are not of nodular form, may be replaced limestone. The dredging of slightly phosphatic limestones which are similar in appearance to these homogeneous pieces of phosphorite lends some support to this suggestion.

ORIGIN OF OÖLITES

Noncalcareous oölites in many deposits have been explained as originally formed of calcium carbonate and later replaced by some other substance. Mansfield (1918, p. 591) at first offered such an explanation for the oölites of the Phosphoria formation. He suggested that the calcium carbonate of the original oölites was replaced by calcium phosphate through the action of phosphatic solutions produced from bacterial decay of marine organisms. He later decided (1927, p. 364) that these phosphatic oölites were probably formed directly from phosphatic colloidal suspensions on the sea bottom through biochemical and physical agencies. As indicated above, most of the sea floor nodules and the

associated sediment contain ovoid oölites. The concentric layering of the oölites indicates a process of accretion, but their oval shape is puzzling in view of the usual spherical shape of oölites.

In some samples oval pellets are found which are composed of finely comminuted limy debris with neither nuclei nor concentric layering. Their appearance suggests that they may have been formed and molded into their present oval shape by being passed through the intestines of marine mud-eating animals. The fine fractions of sediment in which these pellets are found are composed almost entirely of calcium carbonate. Pellets in all stages of phosphatization are present, ranging from those which are composed of unaltered calcium carbonate to those which have been completely phosphatized both by replacement and by filling of interstices.

TIME OF PHOSPHORITE DEPOSITION

GENERAL CONSIDERATIONS

Regardless of the method of formation, a separate problem is involved in determining the time and manner in which the nodules were collected into the deposits in which they were found. Hypotheses must explain two facts of particular significance:

(1) Phosphorite nodules have a wide areal distribution, being obtained at 43 stations off southern California. This material was recovered in greater quantities and from more stations than any other type of rock. Several large hauls consisted almost entirely of these nodules. The wide surface distribution is in marked contrast to the known distribution of phosphorite on land in California or elsewhere. In a region of outcropping beds of phosphorite, such as that of the Permian Phosphoria formation of the Rocky Mountains, the phosphorite forms only a small percentage of all the rocks exposed at the surface. If such an area were submerged intact, there would be little chance that dredging would recover large amounts of phosphorite such as were found off southern California. This implies that the phosphorite was obtained from a surface covering rather than from the outcropping edge of a buried formation; in other words the nodules were dredged from a single horizon rather than from a stratigraphic section.

(2) The nodules contain a predominance of Miocene Foraminifera. Those from 16 stations enclose identifiable Foraminifera, which are dominantly middle and upper Miocene. In nodules from at least one station, a mixed fauna of Miocene and Pliocene Foraminifera is present. In several instances, separate nodules and phosphatic cement of conglomerates contain Quaternary Foraminifera.

POSSIBLE MIOCENE AGE OF PHOSPHORITE

General.—The data given above indicate that at least a small portion of the phosphorite must be Quaternary; however, there remains the problem of whether the bulk of the phosphorite which covers so much of the submerged banks, slopes, and shelves is Miocene as indicated by the foraminiferal content or whether it can be explained by deposition of phosphatic material at a more recent time. There are at least two ways in which the phosphorite could have been formed during the Miocene, and still be abundant: (1) The phosphorite might have formed during the Miocene on submarine highs which have been neither eroded nor covered with sediment since that time; (2) the nodules on the present sea floor may have undergone residual concentration from Miocene rocks.

Both of these explanations appear to be favored by the following points:

(1) Miocene Foraminifera are predominant within the nodules.

(2) The phosphorite containing Miocene Foraminifera is different from that enclosing Quaternary Foraminifera.

(3) Phosphorites on land in southern California are more abundant in rocks of Miocene than in those of other ages but are entirely different from those on the present ocean floor.

(4) Almost all of the phosphorite is associated with nonphosphatic Miocene rocks, whereas the 12 dredgings consisting entirely of sedimentary rocks of other ages lack phosphorite.

(5) Phosphorite is more abundant in dredgings on the shelf and in the canyons adjacent to land where Miocene rocks outcrop.

(6) The transported phosphorite nodules of the Lomita Quarry also contain Miocene Foraminifera, but are found in a lower Pleistocene (or upper Pliocene) formation, and hence were originally pre-Pleistocene.

(7) In the Gulf of California, phosphorite similar to that found off California contains Miocene Foraminifera.

Formed in nondepositional areas persisting since Miocene.—Submerged, nondepositional highs may have developed in the continental borderland area in middle Miocene since this is said to have been a time of widespread faulting and basin development in California (Reed, 1933, p. 286); and they may have remained above the adjacent basins and yet below the surface of the ocean since that time. If this were true, the nondepositional nature of such topographic highs (Shepard, 1941) should have prevented the covering of these surfaces with clastic sediments and phosphorite deposited in the Miocene might have remained uneroded. This hypothesis reconciles the data suggesting *in situ* deposition with the paleontological evidence. Supporting both the suggestion that the banks and

the basins were initiated by basin development in the Miocene and that they have remained essentially nondepositional environments since that time, is the fact that nearly all the nonphosphatic sedimentary rocks dredged from these banks are Miocene. Practically no Pliocene material has been obtained.

However, there are many objections to this hypothesis. The continental borderland off southern California is known to be unstable as shown by the elevated terraces of probable Quaternary age on San Clemente, San Nicolas, and the Santa Barbara Islands. Relatively recent submarine fault scarps are found, as for example in the vicinity of San Clemente Island (Shepard and Emery, 1941, p. 21-25). The presence of phosphorite with Miocene Foraminifera on some of these fault scarps forms a serious objection to the hypothesis under consideration since in all probability the surfaces do not date back to the Miocene. There are insurmountable difficulties in attributing continental shelves such as that of Santa Monica Bay to wave erosion during the Miocene and still more serious difficulties in dating as Miocene the canyons which cut this Santa Monica Bay shelf. Yet both the shelf and the canyon walls have considerable quantities of phosphorite, some of which contains Miocene Foraminifera.

The existence of the outlying banks since the Miocene might have led to the deposition of Pliocene phosphorite on these surfaces, but there is little evidence that nodules of this age are present. It is incredible that extensive deposits of phosphorite formed on banks during the Miocene could have remained uncovered and free from erosion in all the intervening time. Evidence of the elevation of the banks and shelves comes from the presence of canyons with fluviatile characteristics cut into them and from the apparent wave eroded surfaces and the rounded cobbles found on many of these highs.

The Miocene cherts, limestones, and mudstones differ greatly from the glauconitic sediments found on the banks and were presumably deposited in broad embayments. Therefore, the banks must have been initiated, that is, elevated above their surroundings, since deposition of these Miocene rocks. If this were true, the phosphorite should contain more recent faunas than the other rocks obtained in the same dredging, but the available data show that the Foraminifera of the phosphorite are in general older than those of the associated Miocene rocks.

Formed by residual concentration of Miocene phosphorite nodules.— Another hypothesis compatible with a Miocene age of the bulk of the phosphorite is that the nodules were concentrated on submarine surfaces by differential marine erosion of soft Miocene beds containing many phos-

phorite nodules. A similar origin is postulated for the Pliocene phosphorites of Florida (Mansfield, 1940a, p. 412, 414, 415). This process would account for the abundance of the phosphorite even where it is probable that the surfaces have been formed in relatively recent time as in the case of the San Clemente fault slope, the Santa Monica shelf, and the walls of submarine canyons.

There are a number of serious objections to this hypothesis. The great majority of the Miocene strata on land contain relatively few phosphorite nodules either in surface outcrops or in well cores. While nodules have been found in extensive drilling operations in Miocene rocks none like those dredged on the sea floor have been reported. The only nodules of phosphorite like those of the sea floor were found reworked into beds of Pleistocene or uppermost Pliocene age in Lomita Quarry and vicinity. Certainly the marine weathering and erosion of a normal Miocene outcrop of the Los Angeles area would yield very little phosphorite. Examination of pebbles on elevated marine terraces of the Palos Verdes Hills failed to reveal any phosphorite despite the fact that these terraces were cut into Miocene formations. Also, phosphorite was not found in the Miocene of San Clemente and Catalina Islands.

A considerable number of the nodules appear to have been deposited *in situ* and to have remained undisturbed since their deposition. If these nodules are residual concentrations from Miocene deposits, their positions would have been disturbed. Furthermore, the impurities in the nodules are similar to the sediment found on the surfaces of slow deposition where the nodules were discovered. No known Miocene sediments are sufficiently similar to those of the present banks for this resemblance to hold.

Therefore, this hypothesis requires the formation of a different type of Miocene deposit in the area constituting the present sea floor from that which accumulated in the area of the present continent and islands. However, the nonphosphatic rocks containing Miocene fossils dredged from the continental borderland give no indication of an environment different from that suggested by the continental and insular outcrops of Miocene age.

POSSIBLE QUATERNARY (OR PLIOCENE) AGE OF PHOSPHORITE

General.—A few nodules of phosphorite and the matrix of the phosphorite conglomerates contain Quaternary Foraminifera. On the other hand, most of the nodules have Miocene Foraminifera. The two following explanations could account for the Miocene Foraminifera despite a Quaternary age for the nodules.

(1) The phosphorite deposits may have been formed by the infiltration of phosphatic solutions into porous Miocene formations with the replacement of some of the Miocene material.

(2) The phosphorite deposits may have been formed by enclosure of reworked Miocene Foraminifera in nodules formed during the Quaternary.

Because dredging of the continental borderland off southern California has shown the preponderant presence of Miocene rocks, both replaced and reworked material should contain some Miocene Foraminifera. Points favoring a Quaternary or at least a post-Miocene origin are as follows: (1) The predominance of phosphorite over other types of rock dredged from the sea floor off this coast, (2) correspondence of the impurities in the phosphorite nodules with the associated sediments, (3) virtual limitation of the nodules to the present sea floor, suggesting a relation to the present seas.

Formed by infiltration and replacement of Miocene limestone.—Since loosely consolidated porous Miocene formations have been exposed on the sea floor over extensive areas during the Quaternary, phosphate solutions may have infiltered these deposits, completely filling the pore space. Precipitation of phosphorite out of these solutions would have produced a rock with a high percentage of phosphorite. More complete phosphatization would have resulted in replacement by francolite of the Foraminifera and other limy portions of the Miocene rock. Some continental phosphatic deposits have been explained in this way as well as some of the material from Agulhas Bank (Cayeux, 1934, p. 130-132).

This infiltration and replacement may have taken place as the result of contact with sea water locally supersaturated with phosphate. Also, there seems to be considerable evidence that the banks off southern California have been elevated to or above sea level (Shepard and Emery, 1941, p. 16). If the banks were exposed as islands they may have become sites of guano deposition. Since phosphatization of rock underlying guano is known to occur, phosphatic solutions from the guano may have produced some infiltration and replacement.

However, the accretionary layers of most of the phosphorite nodules are highly inconsistent with a replacement origin. Also, available samples of rock, which had been replaced by phosphatic solutions, are quite unlike the material found on the sea floor off California. Therefore, this explanation can apply at most to a small portion of the phosphorite.

Formed in reworked Miocene deposits.—There are numerous Pliocene and Quaternary beds which contain a considerable percentage of Miocene Foraminifera (M. L. Natland, personal communication). Also, at many localities on the sea floor, sands have been encountered which are charac-

terized by an abundance of Miocene Foraminifera. If Miocene Foraminifera were abundant on the sea floor, phosphorite of Quaternary age could incorporate these Foraminifera into nodules. Nodules would also contain glauconite and fragments of underlying formations found on these surfaces of nondeposition. All these impurities are present. It is possible that associated with the Miocene Foraminifera are a few Quaternary forms which have been overlooked because of their scarcity. A mixed assemblage of Miocene, Pliocene, and Quaternary Foraminifera was found in phosphorite from one locality. Small nodules of phosphorite such as the Miocene does contain may possibly have served as nuclei for the growth of the larger nodules. Therefore, the previous existence of phosphorite in the Miocene might have favored further deposition on surfaces containing eroded material of that age.

An objection to this mode of origin is that mixed faunas of Miocene, Pliocene, and Quaternary Foraminifera have not been more commonly identified in the nodules. Furthermore, the nodules frequently contain nests of a single species of Foraminifera, such as a Miocene *Siphogenerina*. Such a nest is difficult to explain if the foraminiferal tests were reworked. In a reworked formation above an unconformity ordinarily there would be an admixture of contemporaneous and older forms but the latter may greatly predominate. The surfaces under discussion are unconformities in the making. Also changes in sea level such as accompanied the Pleistocene or changes in the chemistry of the sea water may have produced conditions which led to the exclusion of Recent Foraminifera from the sediment in which the nodules were formed.

SUMMARY AND CONCLUSIONS

Nodular phosphorite was dredged in great abundance from the sea floor off southern California, some hauls containing hundreds of nodules. These nodules have protuberances and cavities with smooth, glazed, brown surfaces. They are composed of irregular layers of collophane and usually enclose scattered phosphatic oölites, glauconite, and foraminiferal tests. Clastic mineral grains, rock fragments, and phosphatized bones are also commonly present. The nodules agree closely with published descriptions of those obtained from the ocean floor in other parts of the world.

Topographic environment is a most important factor in determining the distribution of the phosphorite. The material has been found in great abundance on the offshore banks and steep slopes and has also been recovered from five submarine canyons and from one area on the continental shelf. The nondepositional nature of the surface on which the nodules are found is indicated by the presence of rock bottom partly covered with a thin patchy veneer of glauconitic foraminiferal sand.

Most of the phosphorite appears to have been formed by direct precipitation from ocean water although it encloses foraminiferal tests and other limy materials which have been replaced by francolite. Layering of the phosphorite indicates discontinuous accretion of collophane. In many cases sufficient time elapsed between periods of accretion to allow the deposition of a coating of manganese oxide. Facts indicative of origin in place include: the great abundance, wide areal distribution, lack of abrasion, large size, poor sorting, and similarity between the enclosed material and the surrounding sediment.

Calculations indicate that ocean water is essentially saturated with tri-calcium phosphate. Hence, slight changes in the physical-chemical environment might be expected to cause the direct precipitation of tri-calcium phosphate on the sea floor. However, the material that is actually precipitated is not tri-calcium phosphate but is a compound or mixture of compounds having the apatite structure.

Foraminifera in a small part of the phosphorite are of Quaternary (or upper Pliocene) age, establishing deposition in relatively recent time. Most of the nodules, however, contain Miocene Foraminifera or resemble other nodules which contain Miocene Foraminifera. While this faunal content may indicate Miocene deposition of the phosphorite the following points suggest a more recent origin:

(1) The deposits are far too extensive to be erosion remnants of a special type of deposition during the Miocene.

(2) The impurities in the nodules indicate deposition in areas where clastic sediments were not accumulating, a condition such as exists now where the nodules are found. The borderland is too unstable to account for the existence of the present surfaces without erosion or deposition since the Miocene. Furthermore, the Miocene formations from the sea-bottom in this area indicate that ordinary deposition was progressing both during and after the time when the faunas found in the phosphorite were living.

(3) The possibility that the nodules are residual concentrates of nodules from the Miocene formations exposed on the banks is opposed by the failure to find similar concretions in the Miocene on land and by the evidence of lack of disturbance of many nodules since deposition.

These considerations lead to the tentative conclusion that the nodules were deposited on the present banks, shelves, and canyon walls during the Quaternary and that previously an abundance of Miocene Foraminifera had been eroded or weathered out of the Miocene formations and concentrated on the surfaces on which phosphorite deposition took place.

REFERENCES CITED

Adler, H., Klein, G., Lindsay, P. K. (1938) *The removal of fluorides from potable water by tricalcium phosphate,* Ind. Eng. Chem., vol. 30, p. 163-165.

Agassiz, A. (1888) *Three cruises of the Blake,* vol. 1, Mus. Comp. Zool., Bull., Harvard College, vol. 14, p. 281-282.

Anderson, J. G. (1895) *Über Cambrische und Silurische phosphoritführende gesteine aus Schweden,* Bull. Geol. Ind. Univ. Upsala, 2, p. 178-200, 220-229.

Blackwelder, Eliot (1916) *The geologic role of phosphorus,* Am. Jour. Sci., 4th ser., vol. 42, p. 285-298.

Cayeux, L. (1934) *The phosphate nodules of Agulhas Bank,* S. African Mus., Ann., vol. 31, p. 105-136 (trans. by S. H. Haughton).

Clark, F. W. (1924) *Data of geochemistry,* U. S. Geol. Survey, Bull. 770, p. 523-534.

Collet, L. W. (1907) *Les dépôts marines,* p. 194-213, Paris.

Dawson, J. W. (1876) *Note on the phosphate of the Laurentian and Cambrian rocks of Canada,* Geol. Soc. London, Jour., vol. 32, p. 285-291.

Emery, K. O., and Shepard, F. P., *Lithology of the sea floor off southern California* (in manuscript).

Galloway, J. J., and Wissler, S. G. (1927) *Pleistocene Foraminifera from the Lomita Quarry, Palos Verdes Hills, California,* Jour. Paleont., vol. 1, p. 35-87.

Gibson, R. E. (1938) *On the effect of pressure on the solubility of solids in liquids,* Am. Jour. Sci., 5th ser., vol. 35A, p. 65.

Goldman, M. I. (1922) *Basal glauconite and phosphate beds,* Science, vol. 56, no. 1141, p. 171-173.

Hendricks, S. B., Hill, W. L., Jacob, K. D., and Jefferson, M. E. (1931) *Structural characteristics of apatite-like substances and the composition of phosphate rock and bone as determined from microscopical and x-ray diffraction examinations,* Ind. Eng. Chem., vol. 23, p. 1413-1418.

Hoots, H. W., Blount, A. L., and Jones, P. H. (1935) *Marine oil shale, source of oil in Playa del Rey field, California,* Am. Assoc. Petrol. Geol., Bull. 19, pt. 1, p. 180.

Irvine, Robert, and Anderson, W. S. (1891) *On the action of metallic (and other) salts on carbonate of lime,* Royal Soc. Edinburgh, Pr., vol. 17, p. 42-52.

Kleinpell, R. M. (1938) *Miocene stratigraphy of California,* Am. Assoc. Petrol. Geol., Tulsa.

Lyman, J., and Fleming, R. A. (1940) *Composition of sea water,* Jour. Marine Res., vol. 3, p. 134-146.

Mansfield, G. R. (1918) *Origin of the western phosphates of the United States,* Am. Jour. Sci., 4th ser., vol. 46, p. 591-598.

——— (1927) *Geography, geology, and mineral resources of part of southeastern Idaho,* U. S. Geol. Survey, Prof. Paper 152, p. 361-367.

——— (1940a) *Phosphate deposits of the United States,* Econ. Geol., vol. 35, no. 3, p. 405-429.

——— (1940b) *The role of fluorine in phosphate deposition,* Am. Jour. Sci., vol. 238, p. 863-879.

Murray, J. (1898) *On the annual range of temperature in the surface waters of the ocean, and its relation to other oceanographical phenomena,* Geogr. Jour., vol. 12, p. 113.

———, and Lee, G. V. (1909) *Depth and marine deposits of the Pacific,* Mus. Comp. Zool., Mem., Harvard College, vol. 38, no. 1, p. 35.

Murray, J., and Reynard, A. F. (1891) *Scientific results, H. M. S. Challenger, Deep sea deposits,* p. 391-400.

Pardee, J. T. (1917) *The Garrison and Phillipsburg phosphate fields,* U. S. Geol. Survey, Bull. 640, p. 224-228.

Reed, R. D. (1933) *Geology of California,* Am. Assoc. Petrol. Geol., Tulsa, Okla.

Revelle, R. R., and Shepard, F. P. (1939) *Sediments off the California coast,* Symposium on Recent Marine Sediments, Am. Assoc. Petrol. Geol., p. 245-282.

Sendroy, J., and Hastings, A. B. (1927) *Studies of the solubility of calcium salts. Part II. The solubility of tertiary calcium phosphate in salt solutions and biological fluids; Part III. The solubility of calcium carbonate and calcium phosphate under various conditions,* Jour. Biol. Chem., vol. 71, no. 3, p. 783-846.

Shepard, F. P. (1941) *Nondepositional environments off the California coast,* Geol. Soc. Am., Bull. 52, p. 1869-1886.

———, and Emery, K. O. (1941) *Submarine topography off the California coast: Canyons and tectonic interpretations,* Geol. Soc. Am., Spec. Paper 31, 171 pages.

Thompson, T. G., and Robinson, R. H. (1932) *Chemistry of the sea,* Nat. Res. Council, Bull. 85, p. 112.

Trask, P. D., *et al.* (1932) *Origin and environment of source sediments of petroleum,* Am. Petrol. Inst., Gulf Publishing Co., Houston, Texas, p. 120.

Twenhofel, W. H. (1932) *Treatise on sedimentation,* 2d ed., Baltimore, Williams and Wilkins Co.

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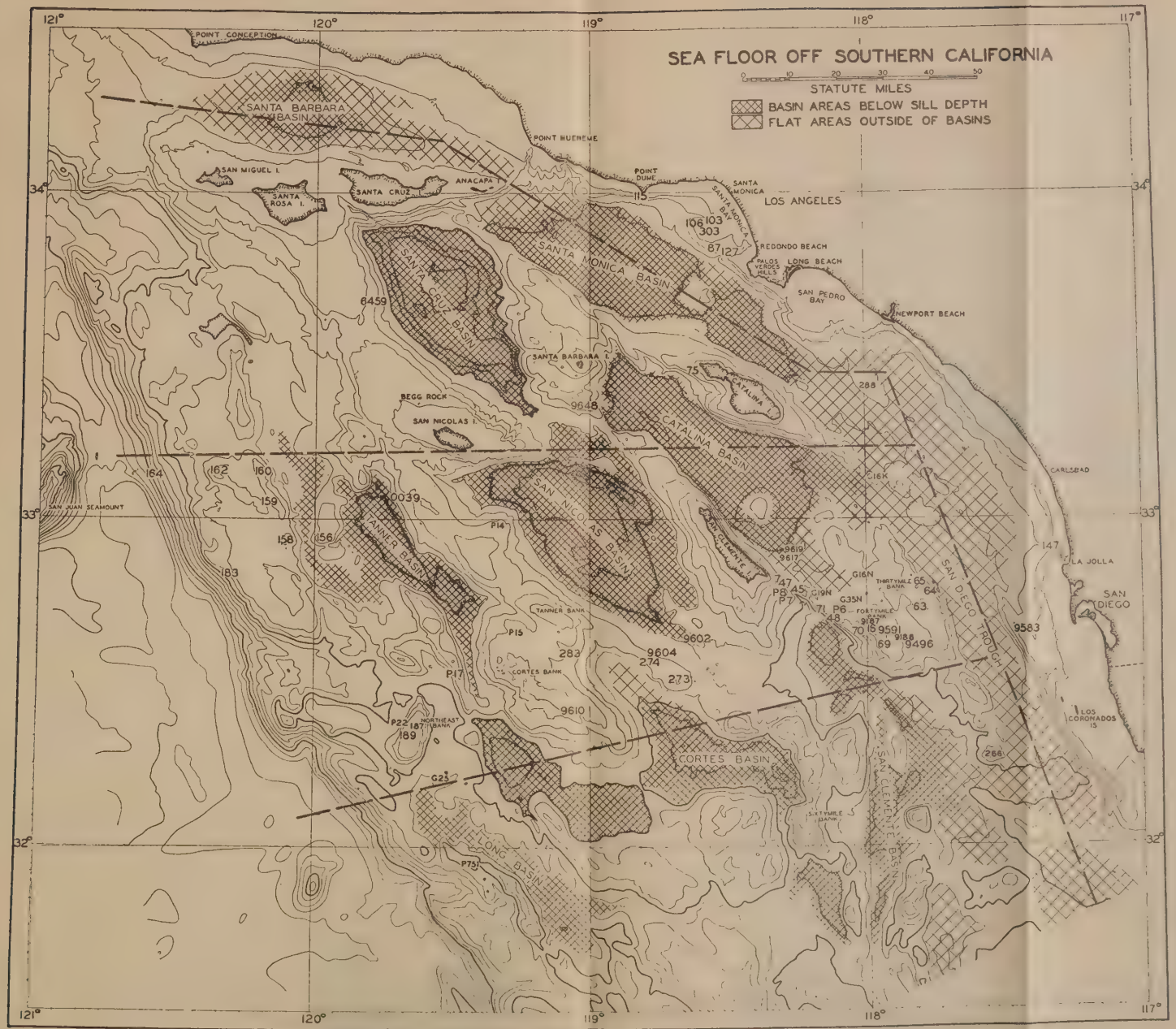

PHOSPHORITE LOCALITIES OFF SOUTHERN CALIFORNIA

Showing the relation of phosphorite to the topographic highs. Contour interval 900 feet with the 300 foot contour added. Large numbers refer to samples containing large fragments of phosphorite and small numbers to samples containing only phosphatic oölites or phosphatic coverings on other rocks. Dashed lines indicate arbitrary divisions of continental borderland related to phosphorite abundance. (*See* text).

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BIOLOGY OF THE CALIFORNIA SEA-MUSSEL (MYTILUS CALIFORNIANUS)

I. INFLUENCE OF TEMPERATURE, FOOD SUPPLY, SEX AND AGE ON THE RATE OF GROWTH [1]

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SIX FIGURES

The object of this study was to determine in a species of marine bivalve living under natural conditions the influence of the available food plankton and temperature on the rate of growth at different ages and at all seasons of the year. For such an investigation the pier at the Scripps Institution of Oceanography is particularly favorable because daily records are taken there of the physical and biological conditions of the water [2]. It is also favorable because the piles of the pier support a vigorous natural growth of this species of mussel.

With the exception of an abstract published by ourselves (Coe and Fox, '41) only a single reference to the observed rate of growth of living individuals of this species has been found and that mentions only the size attained at the age of one year (Scofield, '17). The observations herein reported

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Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 65-1-07-2317.

show that on the coast of Southern California a well-nourished individual may reach a length of 86 mm. at the end of its first year, 120 mm. at the age of 2 years and 140 to 150 mm. when 3 years old. Thereafter the rate of growth is considerably less. In this region the span of life is presumably shorter than farther north, for the largest mussels found here are seldom more than 180 mm. in length, while shells of the same species may reach a length of more than 240 mm. north of Puget Sound.

METHODS

In order to determine any variation in the rate of growth at successive ages, more than 1000 mussels of various sizes were removed carefully from their attached positions on the piles by cutting the byssus strands and placed in separate compartments of large wire-screened boxes suspended from the pier and held at a position from 1 to 2 meters below mean low tide level.

The sizes selected at the beginning of the first year were 10, 20, 22, 26, 30, 40, 50, 60, 70, 90 and 110 mm. in length. The numbers of each size taken for this first experiment are shown in table 2. Each month thereafter every individual was measured and the increase in size, if any, recorded. At the same time a new lot of 10 mm. individuals was added. During the second year the program was varied by the addition of individuals of the various sizes at frequent intervals during the year.

By this method the experimental boxes contained at all times, in separate compartments, representatives of all sizes between 5 and 120 mm. or more in length. These were found to include all ages between 1 month and 2 years of age, as was easily determined by noting the growth of each size from month to month. Consequently any variation in the rate of growth as correlated with age, size, season, food supply, temperature and other environmental conditions could be detected as soon as the results of the various measurements were tabulated each month.

The mussels were exposed to the air only at the time of the monthly measurements. On the adjacent piles, however, most of the natural population of mussels grow at levels having some exposure during low tides.

The California mussel is found only on open coasts, fully exposed to the vigorous surf of the ocean. The question arose as to whether surf action and exposure to the air might be necessary for normal nutrition and growth. The rapid increase in size shown by the experimental mussels indicates that neither of those factors is essential. Consequently there seems to be no reason for believing that periodic exposure enhances the growth rate or is otherwise beneficial except as a protection against sea-stars, fishes, crustaceans and other predatory enemies. The action of the surf may be beneficial in supplying a greater abundance of oxygen than would be found in quiet water. The lower boundaries of the mussel colonies on the piers and rocks appear to be determined by predatory enemies which are less able than the mussel to withstand the effects of exposure. At higher levels the growth rate is evidently more or less retarded, although in some localities it is only at such levels that the mussels are able to withstand the depredations of their enemies.

The investigation was originally planned to extend 1 year only but the first experiment was accidentally terminated at the middle of the twelfth month by a severe storm, during which the hoisting apparatus was broken and the experimental crate lost beyond recovery. Consequently the data for the first year are complete for 11 months only. The growth during the last month of the year has been estimated from that of the 2 preceding months, with the fact in mind that no drastic changes of temperature or available food took place during the final interval.

Partly because of this accident, but more particularly to determine whether the correlations observed between variations in the growth and changes in the environmental conditions for the year 1940 were typical, it was decided to repeat the experiment. The data obtained during this second year,

with a somewhat modified procedure and on a larger scale, have been helpful in understanding the interrelations of growth rate and environment.

PREVIOUS STUDIES ON THE GROWTH OF SEA-MUSSELS

We have found but two papers dealing with the rate of growth in this species. One of these, by Scofield ('17), contains the mere statement that Dr. Harold Heath found that this mussel "grows from the egg to a length of 3½ inches in one year" in Monterey Bay.

The other paper, by Richards ('28), comprises a statistical study of the growth rings in more than 1000 shells but contains no observations on the rate of growth of the living animals. The shell was reported to be "definitely proportioned" at all sizes but it was impossible to correlate the growth rings with age and size.

Studies on Mytilus edulis indicate a generally slower rate of growth than is here reported for the California mussel. Field ('22) estimated the growth rate on the coast of southern New England as about 25 mm. per year for the first 3 years of life and much less thereafter. Coulthard ('29) found that under favorable conditions during the summer at St. Andrews, New Brunswick, young individuals measuring 7 mm. in length showed a maximum increment of 8 mm. in 60 days. The most rapid growth observed was in a 10 mm. individual which showed a gain of 11 mm. in 41 days. This was an average increase of 0.27 mm. per day.

Richards ('35) found that at Woods Hole, Massachusetts, recently settled individuals of M. edulis reached an average length of 19 mm. at the end of their first winter. They were then about 6 to 8 months of age. One year later the average length was 34 mm., indicating an increase of 15 mm. At the end of the following year, when they were about 2½ years of age their average length was 50 mm. The increase, 16 mm., was thus slightly more than during the preceding year. There was very little growth during the coldest months. Growth began toward the end of winter and continued to midsummer,

when it was retarded, with another growing period during the autumn.

At St. Andrews, New Brunswick, Newcombe ('35) confirmed the observation of Mossup ('22) that growth is more rapid when the mussels are continuously or almost continuously submerged than when they are exposed to the air for several hours each day. The mean increase in length during the growing season of the year was 16 mm. in the submerged group and 12.1 mm. in the periodically exposed group. Warren ('36) also found that continuous submergence near the surface of the water resulted in maximum rate of growth. A mean of 7.77 mm. was recorded for the increase during 58 days in summer. It may be noted for comparison that in the California mussel a similar increase is of frequent occurrence in a single month (table 1).

RATES OF GROWTH IN OTHER PELECYPODS

Of the numerous observations on the growth rate of various species, the following may be mentioned as typical examples.

Mya. On the southern coast of New England the soft-shell clam, Mya arenaria, may reach a length of 10 to 15 mm. at the age of 2 months and 40 to 48 mm. when 4 months old. Thereafter the rate of growth is greatly retarded by the lower temperatures of autumn and winter and by the formation of sexual products the following spring. Hence relatively few individuals exceed 50 mm. in length during the first spawning season when about 1 year of age. A length of 60 to 80 mm. may be reached when about 2 years old, but in later life the increment in size is relatively slow. An extreme length of 143 mm. has been reported. For most individuals, however, the rate of growth is much less, some being only 8 to 20 mm. long when spawning at the end of their first year (Coe and Turner, '38). It is also less in more northern localities (Newcombe, '36).

Siliqua. The studies of Weymouth, McMillan and Rich ('31) on the growth of Siliqua patula at widely different latitudes on the Pacific coast demonstrate that the rate of growth is much more rapid in the south than in more northern localities,

although in the former region the life is shorter and the maximum size ultimately attained is less than farther north. On the coast of California a length of about 90 mm. may be attained at the age of 1½ years, while on the coast of Alaska the length at the same age is but little more than one-fourth as great.

Lampsilis. The fresh-water mussels show similar differences in their growth rates in southern and northern localities, as well as different rates in the two sexes (Chamberlain, '31).

Cardium edule. On the southern coast of England this species was found by Orton ('26–'27) to reach a length of about 6 mm. during its first month of growth under favorable conditions. The rate then decreases slowly at first but more rapidly as the temperature falls in the autumn. The length at the end of one season's growth is commonly 20 to 25 mm., with an increment of approximately 10, 5 and 3 mm. during the second, third and fourth seasons, respectively.

RATE OF GROWTH OF THE CALIFORNIA MUSSEL AT SUCCESSIVE AGES

As was to be expected, the younger individuals of our experimental series showed a more rapid increment in length than the older. The mean and maximum increase in each group during each month of the first year of the experiment is shown in table 1. It will be noted that one individual in the 10-mm. group gained 11 mm. in length during April and 12 mm. during May. Although the monthly rate of increase was somewhat lower during the remainder of the year, the total increment was 80 mm. in 11 months. This average of more than 7 mm. per month was the highest yearly average recorded, the mean increase for the fifty-three individuals of the original 10-mm. group being 5 mm. per month (table 1).

In both the 26- and 30-mm. groups there was an average maximum gain of 6 mm. for each of the 11 months of the experiment or a total increase in length of 66 and 67 mm. re-

spectively. The largest increases in the 40-mm., 50-mm., 60-mm. and 70-mm. groups were 56, 47, 49 and 44 mm. respectively.

There was a sharp decrease in the rate of increment in the 90-mm. group as compared with those of the smaller sizes and the difference was even greater in the group which measured 110 mm. in length at the beginning of the experiment (table 1).

If the increase during the twelfth month was equal to that of the preceding month it appears that a 10-mm. individual

TABLE 1

Monthly increase in length, in millimeters.

Upper line, mean; lower line, increased length of longest individual

LENGTH	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	TOTAL INCREASE 11 MOS.	DEC.*	1 YEAR	
														Length*	In-crease*
mm.															
	3*	3*	4	7	9	6	4	2	6	6	5	55	5	70	60
10	6*	6*	7	11	12	7	7	3	7	8	6	80	6	96	86
	3	4	5	8	6	5	4	1	3	5	4	48	4	72	52
20	5	6	5	9	9	6	4	1	4	6	6	61	6	87	67
	3	4	5	5	6	5	4	4	2	3	2	43	2	68	45
22	6	7	7	5	8	8	3	3	3	6	2	58	4	84	62
	7	3	5	6	7	6	4	2	6	5	4	55	5	86	60
26	9	5	5	7	7	7	4	2	6	7	7	66	6	98	72
	5	3	5	6	5	5	4	2	5	5	5	50	4	84	54
30	10	7	8	10	6	4	4	2	6	4	6	67	5	102	72
	5	3	5	7	6	3	3	1	3	4	3	43	3	86	46
40	7	5	6	9	7	2	5	1	5	5	4	56	4	100	60
	4	2	4	5	3	2	2	1	3	3	3	32	2	84	34
50	5	5	6	7	6	3	3	1	4	4	3	47	3	100	50
	4	3	6	2	4	2	2	3	2	2	3	33	3	96	36
60	6	4	6	6	4	4	0	5	2	4	8	49	6	115	55
	5	1	5	4	3	2	2	1	2	3	2	30	2	102	32
70	7	3	5	4	3	3	3	1	5	4	6	44	5	119	49
	4	2	3	2	3	2	1	1	2	1	2	23	1	114	24
90	6	2	3	3	4	3	1	1	2	0	3	28	2	120	30
	0	1	1	1	1	2	1	0	2	2	1	12	2	124	14
110	2	1	1	0	1	1	1	1	3	4	5	20	4	134	24
	43	29	48	53	53	40	31	18	36	39	34		31		
Total	69	52	58	71	67	48	35	21	47	52	52		51		

* Estimated.

may grow to a length of 96 mm. or more and increase its volume from 0.15 to 53.8 cc. in 1 year. This represents an increase of 860% in length and 36,000% in volume (table 2).

A 10-mm. individual may more than double its length in one month (fig. 1) and a 5-mm. individual may become 12 mm. or more in length during the same period. From the observed growth rate of small individuals it may be assumed that a

TABLE 2

Percentage increase of each size group during 1 year.

NO. INDIVIDUALS	ORIGINAL		AT END OF 1 YEAR (TWELFTH MONTH ESTIMATED)			INCREASE					
	Length	Vol.	Length		Vol.	Length				Vol.	
			Mean	Max.		Mean	p. c.	Max.	p. c.	Max.	p. c.
	mm.	*cc.*			*cc.*						
53	10	0.15	70	96	54	60	600	86	860	53.8	36,000
56	20	0.84	72	87	46	52	260	67	335	45.1	5,400
103	22	1.1	68	84	39	45	205	62	282	37.9	3,500
22	26	1.5	86	98	56	60	231	72	277	54.5	3,600
43	30	2.5	84	102	64	54	180	72	240	61.5	2,500
52	40	5.5	86	100	60	46	115	60	150	54.5	990
71	50	9	84	100	60	34	68	50	100	51	570
30	60	16	96	115	85	36	60	55	92	69	430
12	70	24	102	119	102	32	46	49	70	78	330
6	90	48	114	120	104	24	27	30	33	56	117
5	110	80	121	134	148	14	13	24	22	68	80

length of 5 mm. may be reached within a month after metamorphosis. It is evident therefore that under favorable conditions the mussel may become at least 86 mm. long when 1 year of age. From the growth rates of the larger mussels in the experiment it may be concluded that a length of 120 to 129 mm. may be reached when 2 years old and 140 or even 150 mm. at the end of the third year (tables 1, 2; figs. 1, 2, 3, 6). The maximum percentage increase in length ranged from 860 for the 10-mm. group to only 22 for the group of largest size (table 2).

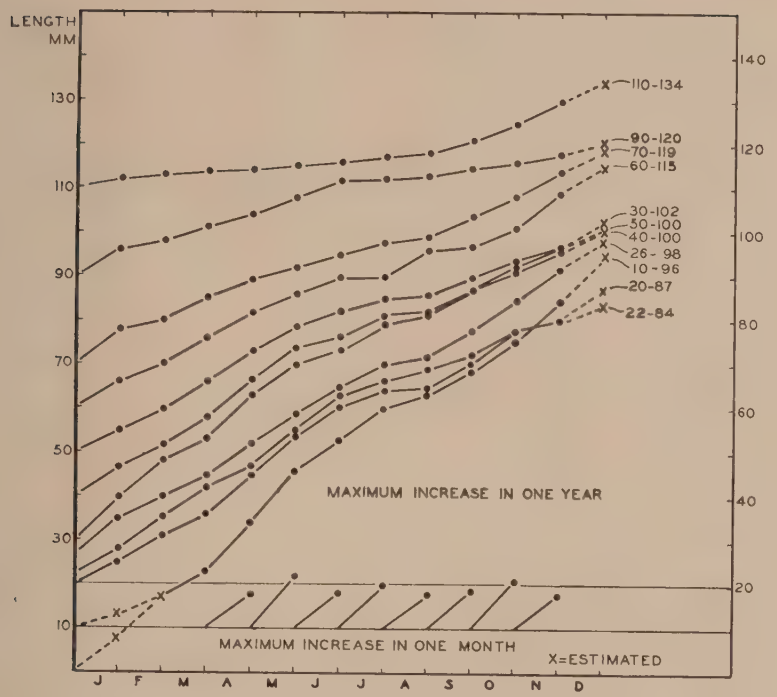

Fig. 1 Maximum monthly increase in length of each size group during the calendar year 1940 (twelfth month estimated). Note decreased rate of growth during midsummer of that year in all except the 60-mm. group. Monthly increases of 10 mm. individuals introduced each month from March to November inclusive are also indicated.

The great variation that was found in the growth rate of different individuals in the same size group is shown in figure 2. It will be noted that for most of the groups the maximum is more than twice as great as the minimum. Hence it seems improbable that the increments in size observed in these experiments represent either the maximum or minimum that may occur in the general population.

During the second year of the experiment the conditions for rapid growth were on the average somewhat more favorable than during the first year. A comparison of figures 4 and 5

will show that there was a less rapid increment in length during the first 5 months and the last 2 months of 1940 as compared with corresponding portions of 1941 but that this is more than balanced by the much greater increase during the summer months of 1941.

Some notable gains of 12, 13, and 14 mm. in 1 month were recorded during the second year but nevertheless no single

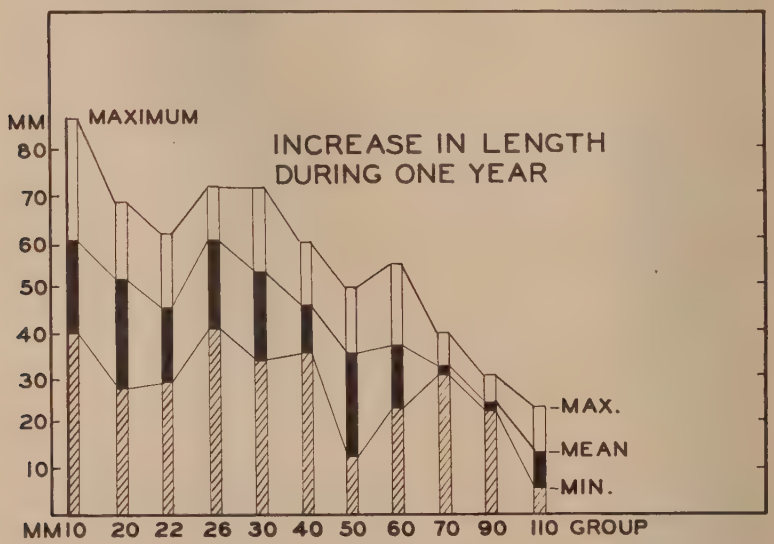

Fig. 2 Increased length of each of 11 size groups during the calendar year 1940 (twelfth month estimated). The total height of the column represents the increased length of the most rapidly growing individual; the top of the black segment the mean increase of the entire group, while the top of the cross-lined segments shows the increased length of the individual with the slowest growth rate. The general trend toward a decreased increment in length with increasing age is evident.

individual in that year made a total increase equal to the maximum reported above for the first year. The months of April and May were favorable for rapid growth in both years. February of both years and December of the second year were unfavorable because of storms and the inflow of fresh water and mud. The low rate of increase during the summer of 1940

is thought to be due largely to the high temperature of the water.

During both years there was also found a monthly variation in the rate of growth for each age group, as shown in figures 4 and 5. Some months in both years proved to be more favorable than others. Allowing for these differences, the approximate average maximum monthly increase in length for successive ages is as follows:

AGE MONTHS	INCREASE DURING 1 MONTH	LENGTH
	mm.	*mm.*
1	4	4
2	6	10
3	7	17
4	8	25
5	8	33
6	8	41
7	8	49
8	7	56
9	7	63
10	6	69
11	6	75
12	5	80

The average maximum increase during each month of the second year of life is approximately: 5, 5, 4, 4, 4, 3, 3, 3, 3, 2, 2, 2 mm., making a total gain of 40 mm. for that year. The maximum gain would be about 1 mm. greater for each month. Hence the maximum length at the end of the second year is about 120 mm. to 129 mm. and the mean 105 mm. if the conditions are favorable (fig. 3).

During its third year the mussel's total increase in length of shell is estimated as only 20 to 30 mm., indicating that the animal when 3 years old will normally measure about 123 to 150 mm. Hence an increment of approximately 80 mm. may be expected for the first year of life, 40 mm. for the second year, 20 for the third and 10 for the fourth (fig. 3).

Increase in volume. The remarkably rapid growth during the first 2 years of life and particularly during the first year is best demonstrated by measuring the increment in weight

or volume. Data for the volumes of each of the 11 size groups used in the experiment are included in table 2 and are shown for all except the larger sizes in figure 6. The largest individual increase in weight and in volume was found in the 70-mm. group. Here the increase in length was only 49 mm.

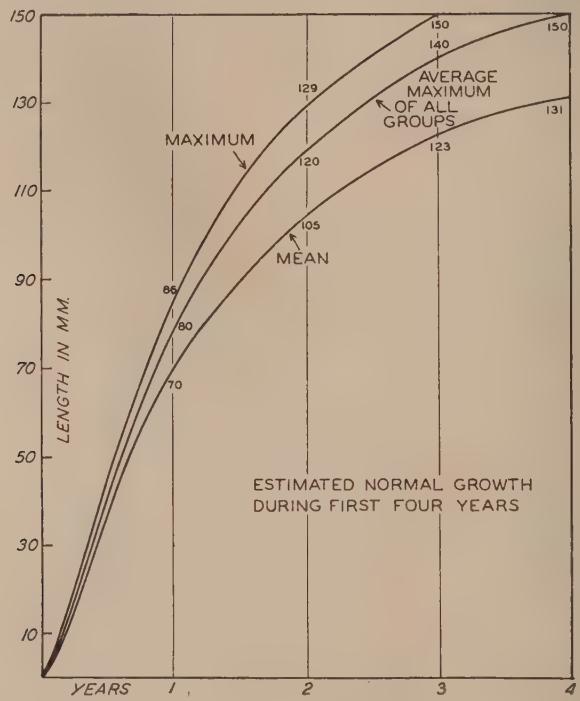

Fig. 3 Estimated normal growth during first 4 years of life; based on the observed growth rates of more than 1000 mussels during the years 1940 and 1941. The maximum is the smoothed curve of the maximum increase in length of the most rapidly growing individuals of approximately the ages indicated. The average maximum of all groups includes the combined data for all seasons of the year. The mean is based upon the means of all groups at all seasons.

during the year but the volume increase was 78 cc. and there was a corresponding increase in weight. The percentage increase in volume ranged from 36,000 for the 10-mm. group to 80 in the group measuring 110 mm. (table 2). The increment in weight and in the chemical substances composing the body will be discussed in a subsequent paper.

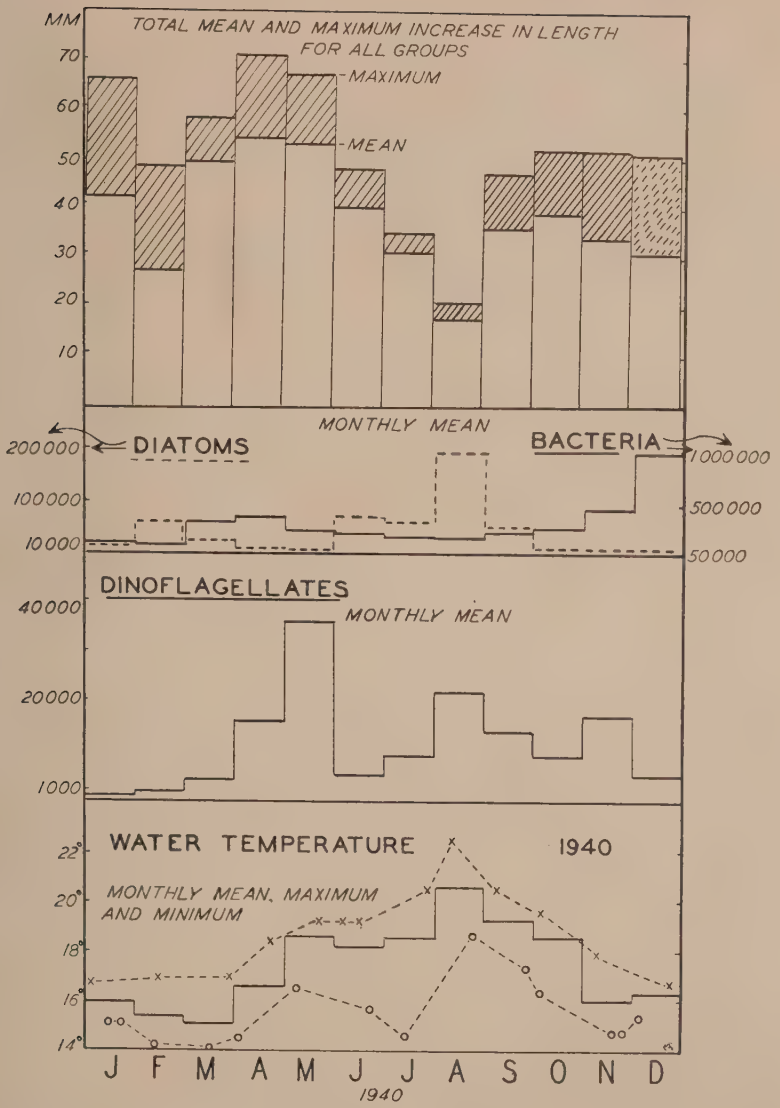

Fig. 4 Comparison between temperature, abundance of phytoplankton (diatoms, dinoflagellates and bacteria) and the combined monthly increment in length of all the size groups during 1940. The minimum rate of growth corresponds with the period of highest temperature. The correlation between growth rate and abundance of dinoflagellates is rather close except for the months of January and August. The numbers indicated for each group of organisms represent the daily average found in one liter of water.

CORRELATION BETWEEN RATE OF GROWTH AND TEMPERATURE

The total increment in length of the 11 size groups for each month of the year as correlated with the surface temperature of the water during 1940 is shown in figure 4. The data show that the growth rate was highest during the cooler months of the year (table 1), with a sharp decrease at the season of highest temperature. During August of that year many individuals made no growth whatever.

During the year 1941, on the other hand, the largest increments in growth occurred during June and July, but again there was a sharp decline in August (fig. 5). In this second year of the experiment the growth rate continued to decrease during the autumn months instead of showing a sharp rise in September as in the preceding year. The higher rate during the summer of 1941 is explained as partly due to the abundance of phytoplankton and partly to the lower and hence more favorable temperature of the water during August.

The optimum temperature for growth in this locality evidently lies between 15° and 19° C., with a sharp decrease in the growth rate above 20°. Richards ('35) observed a similar decrease in the growth of M. edulis at Woods Hole, Massachusetts, in midsummer, when the temperature exceeded 20°. He also found a cessation of growth in the coldest months of the winter, when the temperature is below 5°. Coulthard ('29) likewise found the optimum temperature for the same species to lie between 10° and 20° on the coast of New Brunswick. Although we find this general inverse relationship between growth rate and highest temperature on the coast of Southern California it should not be assumed that the same relations exist in the more northern and cooler portion of the range

Fig. 5 Comparison between temperature, abundance of phytoplankton (diatoms, dinoflagellates and bacteria) and the combined monthly increment in length of all the size groups during 1941. Except for the month of January there is a close correlation between growth rate and abundance of dinoflagellates. The numbers indicated for each group of organisms represent the daily average found in 1 liter of water. The exceptionally low rate of growth in December was partly due to a severe storm lasting several days, during which the mussels were brought into the aquarium.

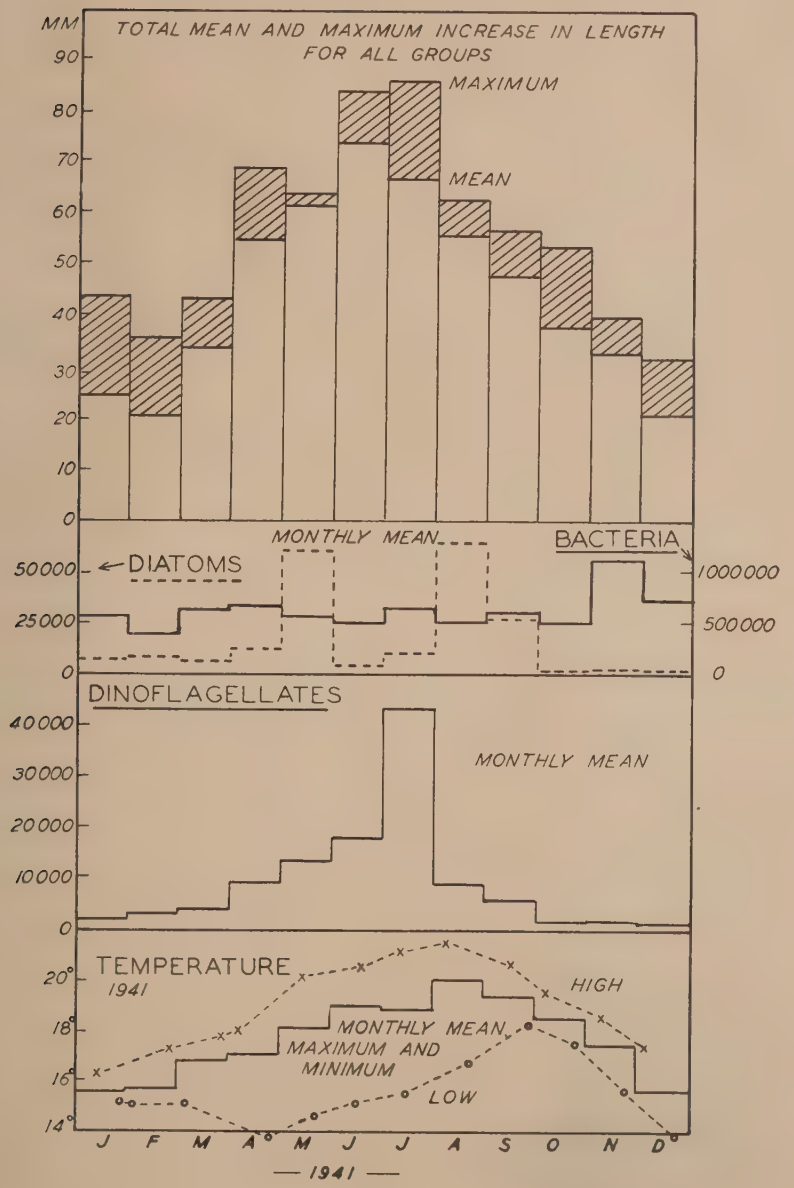
MM
TOTAL MEAN AND MAXIMUM INCREASE IN LENGTH FOR ALL GROUPS
MAXIMUM
MEAN
90
80
70
60
50
40
30
20
10
0
MONTHLY MEAN
BACTERIA
DIATOMS
50000
25000
0
1000000
500000
0
DINOFLAGELLATES
MONTHLY MEAN
40000
30000
20000
10000
0
TEMPERATURE
1941
HIGH
MONTHLY MEAN
MAXIMUM AND MINIMUM
LOW
20°
18°
16°
14°
J F M A M J J A S O N D
— 1941 —

Figure 5

of the species. On the contrary we should expect to find a positive correlation between rise of temperature and growth rate in cooler regions.

The decreased growth rate that we have observed during August of both years was not due entirely to lack of food, for both dinoflagellates and diatoms were abundant throughout the month. Nor was it due to the reproductive processes, for the decrease was as conspicuous in the young individuals as in those that were sexually mature. The conclusion is reached that temperatures above 20° are less favorable for general metabolism in this species of mussel in this locality than those a few degrees lower.

The minimum temperatures shown in figure 4 for February, March, April and July, 1940, and in figure 5 for April and May, 1941, indicate upwelling water. Such upwelling normally contributes to the growth of the phytoplankton and this is ultimately reflected in the growth rate of the mussels.

CORRELATION BETWEEN GROWTH RATE AND FOOD SUPPLY

Fox ('36) and Buley ('36) report that the principal food of the California mussel consists of various species of dinoflagellates when these are abundant in the water. At times these microscopic organisms constitute at least 99% of the organic material in the stomach contents. As many as nineteen species were represented. When dinoflagellates were present in only small numbers the mussel was found to feed mainly on diatoms, including twenty-five or more species, small particles of plant detritus with adhering bacteria, and such other minute particles as may be suspended in the water. The fact that dinoflagellates are selected by the mussel in preference to diatoms is demonstrated by the relative abundance of the two types of organisms in the sea and in the stomach contents. During a period of 7 months Buley ('36) found that there were about forty-eight times as many diatoms as dinoflagellates in the water, while in the stomach contents during the same period there were nearly forty-eight times as many dinoflagellates as diatoms.

MacGinitie ('41) preferred diatom cultures for artificial feeding. ZoBell and Feltham ('38) have demonstrated that the mussel may live for 9 months or more and increase slightly in weight when kept in the dark and fed pure cultures of bacteria only.

The mussel is evidently a general plankton feeder, for the stomach contents show that it ingests at times a vast assemblage of minute organic and inorganic particles and organisms, including in addition to those mentioned above, invertebrate ova, spermatozoa, plant zoospores, flagellates and other protozoa, and various unicellular algae, both living and dead. The food does not consist entirely of these organized cells, however, but includes also the products resulting from the cytolysis of the dead cells of the phytoplankton and algae, as well as of the animal population living in the neighborhood. This detritus, containing chloroplasts, starch granules, oil globules and protein particles, may constitute at times a considerable portion and perhaps the principal portion of the mussel's diet. Minute grains of sand and tiny bits of shell are usually present in the digestive canal. Fox ('36) observed the ingestion of both organic and inorganic particles of no nutritional value, with vigorous rejection of poisonous substances.

It may be recalled that the mussel feeds by secreting over the gills a thin sheet of mucus in which all the particles in the water passing through the gills are collected. The mucus with its contained particles is then drawn into strings and passed forward to the labial palps. The palps thereupon remove any undesirable particles and draw the rest of the mucus string into the mouth (MacGinitie, '41).

Since increase in size must always be dependent upon a sufficient food supply it may be of interest to correlate the rate of growth of the experimental mussels during each month of the year with the relative abundance of three of these sources of food (dinoflagellates, diatoms and bacteria) in the surrounding water at the same time. Such correlations are shown in figures 4 and 5.

Dinoflagellates. Whenever available in sufficient numbers these organisms doubtless represent the basic or principal food supply of this species of mussel. The number present in the water surrounding the mussel beds has been found to vary greatly from year to year, from season to season and even from day to day. Occasionally these organisms become so numerous as to impart a distinctly reddish coloration to the sea. At such times there may be several millions in each liter of water.

During other periods which may extend for weeks or months they are relatively scarce, with populations dropping below 100 per liter. Their abundance during each month of the year 1940 is shown in figure 4 and for the year 1941 in figure 5. In the month of January, 1940, there was a daily average of only 385 dinoflagellates per liter. In February and March the averages were 1362 and 3870 respectively. In April the population per liter increased to over 16,000 and in May to 36,000. A sharp decrease in June was followed by an increase to over 9000 in July and 21,700 in August. During the next 4 months the numbers of these organisms averaged 13,800, 8600, 17,100, and 5400 respectively. The highest daily average for any one week was in May, when there were more than 40,000 in each liter of water. Assuming a rate of water intake into the mantle cavity of 2.6 liters an hour (Fox, Sverdrup and Cunningham, '37), a large mussel may have drawn into its mantle cavity during that week about 2,500,000 dinoflagellates each day. Experiments in this laboratory have shown that the mussel does not feed all of the time when it is actively passing water through the gills. Consequently it is probable that only a portion of these were actually ingested and still fewer assimilated, as was often evident by the great number of intact cells present in the feces.

It is shown in table 2 that a mussel with a shell length of 70 mm. may increase its volume by as much as 78 cc. during 1 year. The dry weight of its tissues may show an increment of 4.2 gr. and the weight of its shell 46 gr. during the same period. The dinoflagellate cells are so minute that in spite

of their great abundance the total supply during the year would not suffice to furnish the mussel with all the material which these increases in volume and mass imply. Consequently, these organisms can be considered as representing only one of the principal food supplies. The supplementary foods must at times constitute the greater part of the diet.

Since the dinoflagellates furnish such a considerable portion of the mussel's food, it is not surprising to find some degree of correlation between the abundance of these organisms and the periods of the mussel's most rapid growth. This relationship is not always exact, however, as may be seen from the graphs in figures 4 and 5. In 1940 (fig. 4) periods of high dinoflagellate production were generally accompanied by an increase in the rate of growth of the mussels. Such was not the case, however, in January, when there was rapid growth in spite of the paucity of dinoflagellates, nor in August when a sharply diminished growth rate accompanied a great abundance of these organisms. Perhaps it may be assumed, therefore, that the mussels obtained sufficient supplementary foods in January, and found the abnormally high temperature of August unfavorable for rapid growth. For the other 10 months of 1940 the correlation between the dinoflagellate population and the growth rate is reasonably close (fig. 4).

For the year 1941 this correlation is even closer. The month of January is again an exception, with a higher growth rate than in February, although the dinoflagellates were slightly less abundant. For the ensuing months both the dinoflagellate population and the growth rate increase to a climax in July and then decrease steadily and rapidly during the remainder of the year, as shown in figure 5. This correlation does not in itself furnish satisfactory evidence that the mussel is necessarily dependent upon dinoflagellates in order to maintain a rapid growth rate but it may prove only that both types of organisms thrive under the same environmental conditions.

Diatoms. The mussel normally ingests only the smaller species of diatoms, the larger and the spiny forms being either prevented from entering the mantle cavity by the straining

device of the mantle edges or discharged from the mantle cavity as pseudofeces.

The diatom population in the water varies greatly from year to year and even from day to day. The numbers found in the vicinity of the mussel beds for each month of the year 1940 is indicated in figure 4. From a daily average of 1400 per liter in January the numbers increased to nearly 36,000 the following month and then gradually decreased until May, when only 700 cells per liter were found. A much greater production followed, culminating in a daily average of nearly 200,000 in August. From that month there was a steady decline through the remainder of the year. The graphs in figure 4 show that the rate of growth in the experimental mussels declined both during the February increase in the diatom population and in the August climax. The fact that the growth rate of the mussels decreased as the diatom population increased is further indication that those species of diatoms which compose the greater part of the population do not contribute greatly to the mussels' food supply.

Again in 1941 there was a decrease in the growth rate during the two periods (May and August) when diatoms were in greatest abundance (fig. 5). And conversely the most rapid growth took place during 2 months (June and July) when relatively few diatoms were present in the water (fig. 5). Both growth rate and diatom populations decreased during the last four months of the year.

Bacteria. Although, as stated above, bacteria furnish the mussel with some nutrition they are not to be considered as one of the major sources of food. Their relative monthly abundance during the 2 years shows but little correlation with the growth rate (figs. 4, 5). During the second year there were about twice as many of these organisms present in the water as during the first year. It may be noted that the mussels showed a somewhat more rapid growth in the year of greater bacterial abundance, implying an increased availability of organic materials for both bacteria and mussels.

RATE OF GROWTH IN THE AQUARIUM

It has been noted frequently that some species of marine plankton-feeding mollusks may live for a year or more in the aquarium without increasing appreciably in size. Others die after a few weeks or months even if abundantly supplied with running sea-water. Failure to grow or to live, as the case may be, has often been blamed on suspected impurities in the water.

To determine the ability of the California sea-mussel to thrive or to survive in the water supplied to the aquaria at this institution 156 individuals of the sizes used in the experiments recorded in this paper, namely those measuring 10, 20, 30, 40, 50, 60 and 70 mm. in length, were suspended in a large aquarium having an abundant circulation of running water.

A few individuals of each group showed a slight increase in length during the first 2 to 3 months but no growth was recorded thereafter. After 5 months only seven remained alive. No deaths occurred during the first month and only six during the second. The greatest mortality took place during the fourth month. The emaciated bodies of the dead and dying mussels showed unmistakable evidence of starvation and only minute quantities of food were found in their stomachs.

That lack of food was one of the principal causes of death was shown by another experiment in which mussels of similar sizes were kept in the same water but were regularly supplied with food. The water in this experiment was aerated and was changed whenever waste products or excess food had accumulated.

The food which was supplied consisted of diatom cultures, finely ground desiccated annelids and mussel tissues, especially gonads, living ova and spermatozoa, and scum from the surface of mud. The stomachs showed that much of this food was ingested.

The growth rate, while not equal to that recorded for most of the experimental mussels living under more nearly normal conditions suspended in the sea, averaged about 2 mm. for each individual during each of the 4 months of this experiment.

The phytoplankton in the water when it reaches the aquarium is much less abundant than in the sea at the pumping station. Less than one-fourth as many dinoflagellates are present. Only when very abundant in the sea does a sufficient number reach the aquarium to supply the mussels with adequate nutriment. At such times the mussels feed actively and sometimes ingest even more of the food organisms than they can assimilate.

The oxygenation of the water in the aquarium is also less perfect than in the sea and deleterious decomposition products and metallic poisons are doubtless present to some extent. Only certain of the most hardy invertebrates thrive for long periods in the aquarium water.

INFLUENCE OF REPRODUCTION ON RATE OF GROWTH

The sexes are separate although, as in many other dioecious species of pelecypods, hermaphroditism is found occasionally. The numbers of the two sexes are equal and there is no indication of a change of sex.

Examination of the gonads of many individuals of different sizes at all seasons of the year has shown that the approach of sexual maturity is already indicated when the individual has attained a shell length of about 25 mm. This would indicate an age of approximately 4 months (fig. 3). At that time the gonadal follicles have reached the mantle and the proliferation of the germ cells is in progress.

Non-motile spermatozoa of nearly normal appearance are often present in males 30 mm. in length and the gonads of nearly all individuals of both sexes more than 40 mm. long contain some apparently mature gametes. During the following 6 to 8 months the gonads continue to increase in size as the body becomes larger, and no satisfactory evidence of spawning in either sex has been obtained until the individual has reached a length of at least 70 mm. That would indicate that the first spawning occurs near the end of the first year if the environmental conditions are favorable.

It was mentioned in the chapter on growth at successive ages that increment in length begins to decrease at the age of about

8 months or when the shell has reached a length of approximately 56 mm. This is shown in tables 1 and 2. This period in life corresponds with the time when the gonads are increasing most rapidly. The growth rate continues to fall until the gonads are fully ripe and spawning occurs. This may take place at any season of the year on the coast of Southern California but occurs most commonly in spring and autumn and least frequently at midwinter and midsummer.

Immediately before spawning the gonads may increase to such a size that their dry weight in either sex may equal or exceed the combined weight of all the other tissues of the body, exclusive of the shell. At that time and during the spawning period there is often a sharp decline in the growth rate, which is indicated by a thickening of the edge of the shell and is permanently recorded as a "growth ring." Following spawning an increased growth rate is temporarily resumed.

GROWTH RINGS; DISTURBANCE RINGS

It is evident that in the mussel, as in many other organisms, growth is intermittent, with periods of rapid increase in size alternating with periods of disturbance or delayed growth. This rhythmical tendency in the growth rate is influenced both by the reproductive processes and by environmental conditions, including temperature, food supply, storms, and exposure to air between tides.

The effect of depositing new shell intermittently is shown a few weeks after metamorphosis by the formation of faintly marked rings on the shell. As many as five rings may be formed in a month, indicating as many periods when the growth was checked. These are often only 1 to 2 mm. apart. During the first year, while the shell is reaching a length of about 80 mm. as many as forty or more rings may be deposited. Several of these are usually much more conspicuous than the others and indicate periods of greater disturbance.

The shells of the experimental mussels faithfully record in their growth rings the history of the experiment. The disturbance entailed in cutting the byssus and in handling the

mussels during cleaning and measuring usually results in the deposit of a more or less distinct ring. The disturbance causes the withdrawal of the mantle from the edge of the shell and when growth is resumed after reaching the sea the new layers of prismatic shell and periostracum are not directly continuous with the old. Since the new growth in such cases underlies the old, the latter will then be more or less distinctly raised as a growth ring. The major rings indicate longer periods of arrested growth or of more disturbing conditions of internal or external origin. Similar disturbance rings have been reported by Orton ('27) in Cardium, Coulthard ('29) in Mya and by others in fresh-water mussels.

Thus the shell at the end of the experiment shows not only the size which the mussel had reached when placed in the experimental box but also the number of monthly examination periods to which it has been subjected. The amount of growth during each month as thus indicated on the shell corresponds rather closely with the records made at the times of the monthly examinations. Some of the shells were marked by notches in the edge in order to show the position of the ring the following month. The monthly rings are often separated by one or more less conspicuous rings not indicated on the inner face of the shell. These likewise indicate interruptions in the growth of the shell due, perhaps, to temporarily unfavorable conditions or to inherent intermittent growth tendencies.

Shells of mussels taken directly from their natural habitat in the ocean also show major and minor growth rings, and these are sometimes very similar to those on the experimental mussels but generally they are much more irregular in their spacing. Storms, exposure and high temperatures may be thus recorded on the shell.

The culmination of gametogenesis and the subsequent acts of spawning may likewise result in the formation of a major growth ring. Many shells show evidence that such rings are produced at intervals of 6 to 8 months, following the first spawning period. Perhaps this may imply that spawning in

many mature individuals may take place at corresponding intervals in this locality.

The annual rings which are characteristic of many kinds of mollusks in more northern localities and which are indicative of diminished or arrested growth during the winter are not formed by the mussel on this part of the coast. Cessation of growth during August of years when the temperature exceeds 21° may be recorded by a ring.

RATES OF GROWTH IN THE TWO SEXES

In some species of pelecypods, including fresh-water mussels, the males usually become sexually mature at a somewhat earlier age than the females. This also occurs in the California mussel. Furthermore, the females of some species are larger than males at the age of 1 year (Coe, '38). Chamberlain ('31) found that males of the fresh-water mussel Lampsis grow somewhat faster than females during their second year, while the females increase more rapidly in size during the following 5 years. It seemed desirable therefore to determine whether there is a sexual differentiation in the growth rate of the California mussel.

A considerable variation in the increase in length of the constituent members of each group was invariably found when the monthly measurements were taken, although all individuals in the group were of approximately the same age. When the sexual condition of each individual was determined at the end of experimental periods of 9 to 12 months it was quite evident that the increased length of the males averaged about 5% greater than that of the females at all sizes above 60 mm. or after 9 months of age. This superiority of the males increased from sexual maturity to the age of 1½ years. It is not known whether the trend is later reversed.

RELATIVE DIMENSIONS OF SHELL AT DIFFERENT AGES

The shell usually retains a close approximation to its original relative dimensions during its first year or until a length of about 80 mm. is reached. Thereafter there is a general

tendency toward a relative increase in width as compared with height (fig. 6).

The relative proportion of height to length decreases gradually as the length increases, being approximately 0.5 for the smaller sizes and 0.4 for the larger individuals (fig. 6). The proportion of width to length, on the contrary, remains nearly constant at all sizes below 140 mm., the ratios being about 0.37

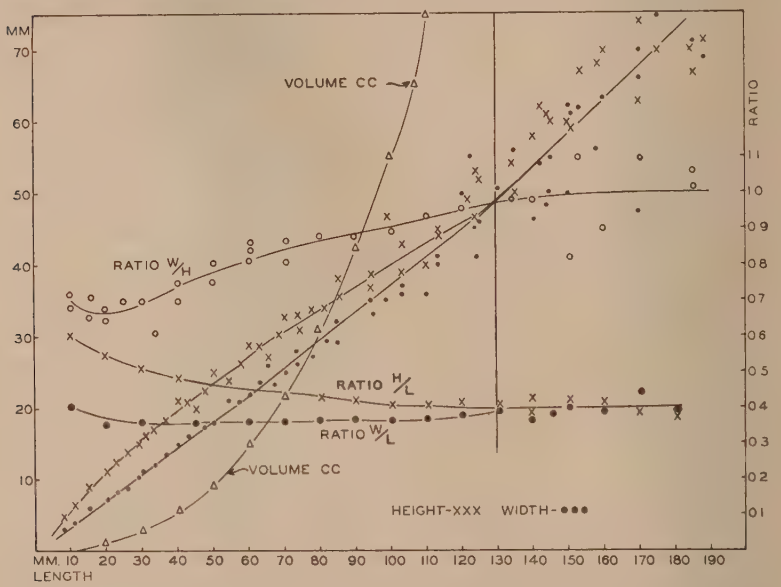

Fig. 6 Relative increase in volume, height and width as compared with length of shell. It will be noted that for sizes less that 130 mm. in length the height generally exceeds the width, while for larger sizes the relative proportions of the shell show great individual variability but the average measurements of height and width are approximately equal. The ratios of width (W) to height (H), height to length (L) and width to length are also plotted.

for lengths up to 60 mm., 0.36 for those 60 to 120 mm., and 0.4 or more for the larger sizes, where the width often equals or exceeds the height. The ratios of width to height consequently increase with the length, being about 0.7 for the sizes up to 30 mm. and increasing to 0.9 at 100 mm. and approximately 1 at larger sizes (fig. 6).

Attention may be called to the fact that large individuals of this species growing on the piles are generally higher and narrower and have thinner shells than those of the same sizes growing on the rocks less than 1000 meters distant.

An exponential increase in water capacity of the gill chamber with length was found by Fox, Sverdrup and Cunningham ('36). Figure 6 shows a similar exponential relationship between length and volume of the whole animal. Logarithmic values of length plotted against logarithmic values of volume, weight of shell, or weight of dried tissue, yield straight lines.

SUMMARY

1. The experiment extended over 2 years, during which time monthly measurements were taken of more than 1000 individuals, representing 12 size groups from 5 to 110 mm. in length. These measurements demonstrate that on the coast of Southern California the California sea-mussel may reach a length of at least 86 mm. within 1 year from the end of the larval stage. The length at the end of the second year may become 120 mm. or more and at the end of the third year 140 to 150 mm. The curve of growth indicates that the yearly increment in length after 3 years of age is relatively small on that part of the Pacific coast.

2. The maximum relative increase in length in the 10-mm. group during the year 1940 was 860% and the increase in volume 36,000%. As the mussel becomes older its relative increment in size decreases until in the third year the maximum increase observed was 24 mm., or 22%, in length and 68 cc., or 80%, in volume.

3. An inverse relationship between rate of growth and increase in temperature was found in 1940, when there was rapid growth during the cooler months of the year with very little growth in midsummer, after the temperature had risen to more than 20° C. In 1941 the maximum summer temperature was lower and the growth rate at this season higher than in 1940.

4. The basic food of this species of mussel consists of various species of dinoflagellates when these are sufficiently abundant, supplemented by a great variety of other minute organisms and organic particles, including small diatoms, bacteria, zoospores, minute ova, spermatozoa, flagellates and other protozoa and unicellular algae, as well as detritus consisting of particles from the cytolysed cells of a great variety of plants and animals.

5. During both years there was a close correlation between growth rate of the mussels and abundance of dinoflagellates in the water. Exceptions were found in January of both years and in August, 1940.

6. Mussels kept in the running water of the aquarium showed little, if any, growth and a high mortality due to lack of suitable food. Others in the same water but plentifully supplied with food grew about half as fast as the mussels living under natural conditions in the sea.

7. Males become sexually mature at an earlier age than females and increase in size more rapidly during the first $1\frac{1}{2}$ years.

8. When the mussels are not densely crowded, the ratio of width of shell to length tends to remain constant at all sizes up to 130 mm. in length, while the ratio of height to length decreases. In large individuals the width often exceeds the height.

9. Growth, or disturbance, rings were formed following each temporary removal of the mussels from the water for measurement. A major ring is often associated with each spawning period.

POSTSCRIPT

Since the foregoing article was set in type the writers have learned of a paper on fouling organisms by Dr. Claude E. ZoBell printed in Circular 588 of the Scientific Section of the National Paint, Varnish and Lacquer Association, 1939. This paper contains a graph showing the "increase in the shell length of mussels attached to submerged experimental blocks and the mean surface temperature of the sea

water at La Jolla during different months of the year." The text in reference to the mussel states only "that the period of the maximum rate of growth of the mussels precedes by a few weeks the maximum water temperature is probably due to the seasonal abundance of foodstuffs in the water and this in turn is influenced by a multiplicity of other inter-related factors besides temperature." No mention is made of the size or the number of individuals measured.

LITERATURE CITED

BULEY, HORACE M. 1936 Consumption of diatoms and dinoflagellates by the mussel. Bull. Scripps Inst. Oceanog., Tech. Ser., vol. 4; pp. 19–27.

CHAMBERLAIN, THOMAS K. 1931 Annual growth of fresh-water mussels. Bull. U. S. Bur. Fish., vol. 46, pp. 713–739.

COE, W. R. 1938 Primary sexual phases in the oviparous oyster (Ostrea virginica). Biol. Bull., vol. 74, pp. 64–75.

COE, W. R., AND H. J. TURNER, JR. 1938 Development of the gonads and gametes in the soft-shell clam (Mya arenaria). J. Morph., vol. 62, pp. 91–111.

COE, W. R., AND D. L. FOX 1941 Influence of temperature, food supply and age on the rate of growth of the California sea-mussel (Mytilus californianus). Anat. Rec., vol. 81, Suppl., p. 127.

COULTHARD, H. S. 1929 Growth of the sea mussel. Cont. Canadian Biol. and Fish., vol. 4, pp. 123–136.

FIELD, IRVING A. 1922 Biology and economic value of the sea mussel (Mytilus edulis). Bull. U. S. Bur. Fish., vol. 38, pp. 127–259.

FOX, DENIS L. ET AL. 1936 The habitat and food of the California sea mussel. Scripps Inst. Oceanog., Tech. Ser., vol. 4, pp. 1–64.

FOX, DENIS L., H. U. SVERDRUP AND JOHN P. CUNNINGHAM 1937 The rate of water propulsion by the California mussel. Biol. Bull., vol. 72, pp. 417–438.

MACGINITIE, G. E. 1941 On the method of feeding of four pelecypods. Biol. Bull., vol. 80, pp. 18–25.

MOSSUP, B. K. E. 1922 The rate of growth in the sea mussel, Mytilus edulis L., at St. Andrews, N. B., Digby, N. S., and in Hudson Bay. Trans. Roy. Canadian Inst., vol. 14, pp. 3–22.

NEWCOMBE, CURTIS L. 1935 A study of the community relationships of the sea mussel, Mytilus edulis L. Ecology, vol. 16, pp. 234–243.

———— 1936 A comparative study of the abundance and the rate of growth of Mya arenaria L. in the Gulf of St. Lawrence and Bay of Fundy regions. Ecology, vol. 17, pp. 418–428.

ORTON, J. H. 1927 On the rate of growth of Cardium edule. J. Mar. Biol. Assoc., vol. 14, pp. 239–279.

RICHARDS, OSCAR W. 1928 The growth of the mussel Mytilus californianus. Nautilus, vol. 41, pp. 99–101.

———— 1935 The growth of the mussel, Mytilus edulis, at Woods Hole, Massachusetts. Anat. Rec., vol. 64, Suppl. p. 68.

SCOFIELD, N. B. EDITOR 1917 Commercial Fishery notes. Calif. Fish and Game, vol. 3, pp. 34–36.

WARREN, A. EMERSON 1936 An ecological study of the sea mussel (Mytilus edulis Linn.) J. Biol. Bd. Canada, vol. 2, pp. 89–94.

WEYMOUTH, F. W., H. C. MCMILLAN AND WILLIS H. RICH 1931 Latitude and relative growth in the razor clam, Siliqua patula. J. Exp. Biol., vol. 8, pp. 228–249.

ZOBELL, CLAUDE E., AND CATHARINE B. FELTHAM 1938 Bacteria as food for certain marine invertebrates. Sears Found., Jour. Marine Research, vol. 1, pp. 312–327.

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INFLUENCE OF NATURAL AND EXPERIMENTAL CONDITIONS IN DETERMINING SHAPE OF SHELL AND RATE OF GROWTH IN GASTROPODS OF THE GENUS CREPIDULA [1]

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EIGHT TEXT FIGURES AND TWO PLATES (THIRTY-EIGHT FIGURES)

Incidental to the study of the sexual transformation which takes place in all normal individuals of the species of Crepidula, the writer has had under observation large numbers of each of seven species of that genus. For each of these the rate of growth and the ready response of young and older individuals to a changed environment have been studied experimentally.

Gould ('17) called attention to the rapidity of growth that sometimes occurs in C. plana. He found that young individuals might grow to a length of 10 to 15 mm. within 2 months during the summer. Ishiki ('36) also mentions that an increment in length of 11 to 16.5 mm. in 2 months is possible during the sexual transformation of C. walshi on the coast of Japan.

Only a few direct observations on the rates of growth of other marine gastropods under natural conditions have been reported. More frequently the rate has been assumed by a comparison of the sizes of the individuals constituting the population at different periods of time (Pelseneer, '35).

Russell ('09) found that marked individuals of Patella vulgata on the coast of Scotland became 20 to 25 mm. in length

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when 1 year of age, 38 mm. at 2 years, 43 mm. at 3 years and 45 to 48 mm. when 4 years old. On the south coast of England the rate of growth of the same species is evidently much greater, for Orton ('28) records a maximum length of 35 mm. at the age of 1 year and 53 mm. at the age of 2 years.

Three of the species of Crepidula living on the Pacific coast, C. onyx Sowerby, C. nummaria Gould and C. lingulata Gould, have widely deviating growth forms. Three other species, C. fornicata Linn., C. plana Say and C. convexa Say, which are common on the southern coast of New England, show similar differences in size, shape and color under varied environmental conditions, as Conklin (1897, 1898) reported more than 40 years ago.

For each of these six species there has been some question as to whether the typical and deviating forms might not, in fact, represent separate species or at least separate varieties. This question has now been answered in the negative, since in each species it has been found possible to change the young of the typical form into the deviating form experimentally. A seventh species, C. adunca Sowerby, living on the Pacific coast, shows less response to a changed environment.

METHODS

For most of the experimental studies on the rate of growth under various conditions, as well as on the changes in the shape of the shell, the young animals were persuaded to attach themselves to the sides of bottomless glass jars and other objects which were then placed in wire-screened boxes and immersed in the sea. Each individual was examined at frequent intervals during periods varying from a few weeks to 1 year. All aspects concerning the lives of the animals, including the changes involved in the transformation from male to female phase and the periods of ovulation and incubation, as well as the rate of growth, were readily observable through the glass walls of the jars. Shells, pebbles and other objects of various shapes and sizes were also included in order to determine their influence

on the sizes and shape of the Crepidulas which became attached to them.

It was found that none of the species experienced normal growth and reproduction either in the running water of the aquarium or in aerated water with artificial feeding. Individuals of three of the species lived for several months to a year under such conditions but increased only slightly in size.

C. adunca, C. nummaria and C. convexa are incubatory, the eggs and larvae being retained beneath the body of the parent until metamorphosis to the adult form has been completed. The four other species produce much smaller and more numerous eggs which develop into free-swimming veliger larvae.

SEXUAL TRANSFORMATION

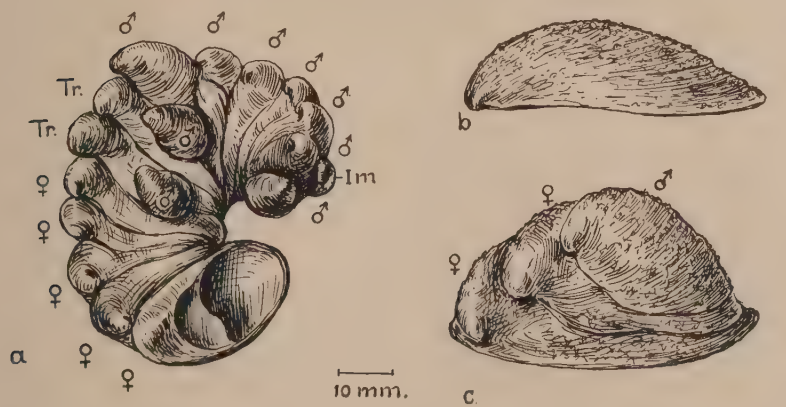

Fig. 1 C. onyx; *a*, growth form onyx; large group consisting of seventeen individuals from intertidal mud flat; the group includes five basal females, two transition individuals and seven superimposed functional males, with two small supplementary males and one sexually immature young (Im); *b*, large, isolated female of the roughened, flattened form (rugosa); *c*, growth form rugosa attached to flat surface; two females with superimposed male.

In all the species of the genus of which the reproductive processes are known all normal individuals function first as males and later change to the female phase. Young individuals in the male phase are motile but older individuals in the female phase are more or less strictly sedentary. Nevertheless the male

when attached in the mating position near the anterior right dorsal margin of the female's shell (text figs. 1 to 7) may retain that position for several months or even for more than a year and long after the individual has transformed to the female phase (Coe, '36, '38a, '38b).

In all of these species the male phase becomes functional under normal conditions whether or not the young animal has an opportunity of associating with other individuals. But the extent of the male phase depends upon such association. If isolated for a period of 1 to 4 weeks (the time depending upon the species), the male-phase individuals usually return to the condition of sexual immaturity which is normally followed by transformation to the female phase. An occasional male-phase individual of C. onyx or C. fornicata of persistently masculine nature, however, may retain that phase for several months after isolation (Coe, '38a, '38b).

GROWTH FORMS

In order to protect the body, the edges of the shell must always be closely fitted to the object to which the foot is attached.

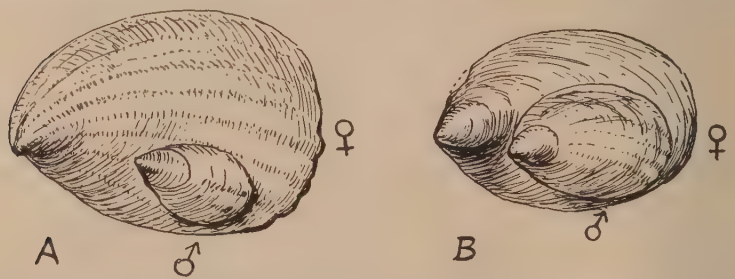

Fig. 2 C. onyx. *A*, mated female with crenulated shell grown upon shell of Pecten. *B*, female with male transferred from shell of Pecten.

Consequently as the shell increases in size by growth from the mantle edges its shape must conform to any curvature or irregularities of the surface to which it is attached (text fig. 2). It may be flat, arched, concave, scalloped or twisted, because of the ability of the animal to adjust itself to objects of various shapes.

The young in the male phase, being motile, have some freedom of choice but the same individuals on reaching the female phase (with the exception of C. convexa and C. adunca) become immovably attached to the substrate.

It is obvious that the ultimate size that may be attained is likewise dependent upon the size of the object to which the foot is attached. If the object be too small or the space too limited for normal growth only dwarfs are possible. In such dwarfs, as Conklin (1898) has shown, the cells of the body and the eggs produced are of normal size but the numbers are less.

C. onyx and C. fornicata

In both these species, one living on the Pacific coast and the other on the Atlantic, two widely different growth forms with

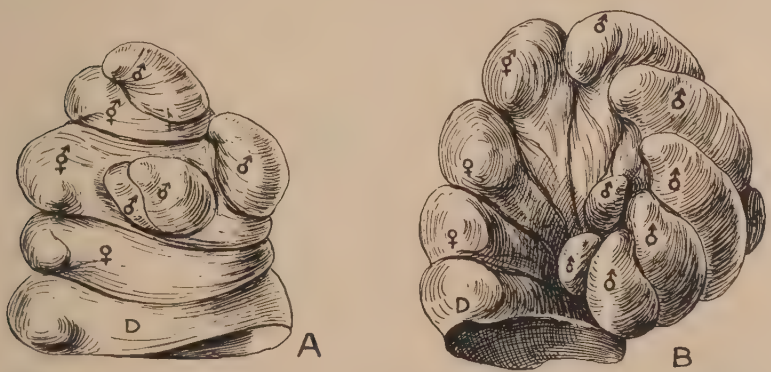

Fig. 3 C. fornicata. *A*, Small group consisting of one dead shell (D), one female, two individuals in process of sexual transformation and four males. *B*, Larger group with two females, one transforming individual and seven males.

many modifications occur in nature. The narrow growth form is found in muddy or sandy localities, as the result of one individual attaching itself upon another of the same species until compact groups of six to sixteen or more are formed (text figs. 1a, 3). Each group maintains itself from year to year by the addition of young male-phase individuals which may settle at the top or side as rapidly as the old females die at the bottom.

Each of the constituent shells is smooth, narrow and highly arched, due to the narrow area available for the attachment of the foot (pl. 1, fig. 5).

The other common growth form has a flat, oval shell and is usually attached singly or in groups of two or three to a surface which allows unrestricted space for growth (text fig. 1, b, c). Consequently the shell may reach a size several times greater than is possible in large groups where the basal shell is small. The individuals shown in figures 5 and 14 (plate 1), although differing so greatly in size and shape, are of approximately the same age.

The narrow growth form is readily changed to the broad form by transferring the young or half-grown individual to a flat surface. If intermediate sizes be used the most bizarre combinations of narrow and broad forms may be produced (pl. 1, figs. 8 to 13; text fig. 8). Numerous other growth forms,

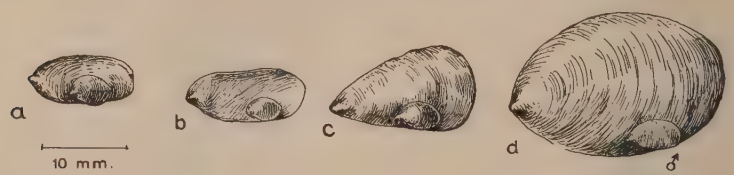

Fig. 4 C. nummaria. *a*, *b*, mature females, dwarf form, with mated males, living in small univalve shells; *c*, *d*, broad form with males attached in mating position; grown on surface which allowed unlimited space for increase in size.

differing in size, shape, color and texture, are found in nature. These testify to the ready response of the young mollusk to varied environmental conditions.

If attached singly to a very small shell, pebble or other object, a highly arched dwarf form results. This may have a dwarf mate and produce a small number of eggs of normal size.

C. plana and C. nummaria

Deviations in the growth forms and sizes of these two species is even more pronounced than in any others of the genus. Both have similar habits, living most commonly within the shells of other gastropods which are either empty or occu-

pied by hermit crabs; but they are also found upon the exterior of various mollusk shells and upon rocks and other objects.

The young often become attached to the interior of such small shells that only narrow, dwarf forms are possible (pl. 2, figs. 16, 16a; text fig. 4, a). But if attached to an object which allows adequate space for normal growth, the shell becomes broadly oval and fully twenty times as large as the more common dwarf form (pl. 2, figs. 19 to 27; text fig. 4, d; text fig. 5, B).

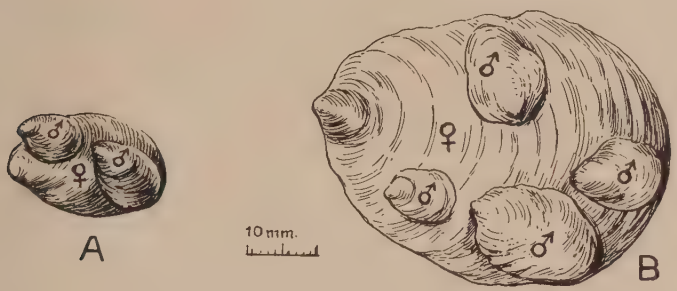

Fig. 5 *A*, C. convexa, female with one mated male and one supplementary male. *B*, C. plana, broad growth form; female with one mated male and three supplementary males.

C. lingulata

This species is likewise responsive to the size and shape of the object to which it is attached. If on a small shell or pebble it assumes a highly rounded or broadly conical form (pl. 2, figs. 29, 29a). On a smooth stone or other object it is flattened, circular and smoothly rounded (pl. 2, figs. 30, 33, 35, 37, 38; text fig. 6). In the crevice of a rock it is flat and irregular in outline (pl. 2, figs. 32, 34).

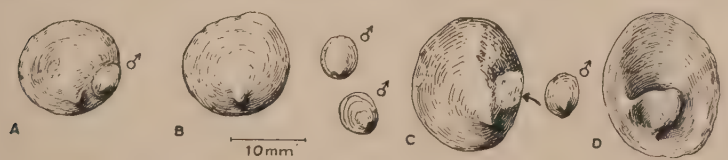

Fig. 6 C. lingulata; shape of shell when grown on unrestricted surface. *A*, female with male attached in mating position; *B*, *C*, females with associated males; *D*, empty shell from ventral surface.

C. convexa

When living on the outer surfaces of small snail shells individuals of this Atlantic coast species are highly rounded (text fig. 5, A) and dark brown in color. On flat stones they become broad and flat. On light colored oyster shells they are likewise flat and so much lighter in color that such growth forms were long thought to represent a separate species.

C. adunca

This species, found on the Pacific coast, has habits similar to those of C. convexa. The highly arched shell (text fig. 7) shows less widely deviating growth forms than any of the foregoing species.

Fig. 7 C. adunca. *a*, female with small mated male; *b*, female with large mated male; *c*, female with one mated male and one supplementary male; *d*, two attached females, each with mated male.

RATES OF GROWTH

Since the shell in Crepidula covers only the dorsal and posterior parts of the body, the ultimate dimensions of the shell can never greatly exceed the area of the surface to which the foot is attached. Hence many individuals fasten themselves to objects so small that the maxium size characteristic of the species can not be attained. Consequently such individuals must remain as dwarfs throughout life.

Individuals of the same age may thus differ greatly in size. Furthermore, under unfavorable growth conditions, such as are often found in the aquarium, the individual may live for a year or more without any considerable increase in dimensions. Normally, however, the sexually immature young grow

rapidly, then the rate of growth decreases in the functional male phase but increases again during the period of sexual transformation, followed by a decrease in the female phase.

Isolated male-phase individuals grow more rapidly than mated males, for the reason that isolation inhibits spermatogenesis and induces the period of sexual transformation. This was also found to be the case in C. walshi (Ishiki, '36).

The normal growth rate at various ages was determined for each of the seven species by observing the increment in size of individuals which had become attached to glass jars and other objects, large and small, immersed in the sea.

C. onyx

It was found that under the most favorable conditions in the sea young individuals of this species increased in length at the rate of 3 to 5 mm. a month during the 2 to 3 months required for the completion of the male phase. During the period of sexual transformation the rate was increased to 6 to 8 mm. in 1 month (text fig. 8).

In the early female phase there was likewise an increment of 4 to 6 mm. in length during each of the first 3 to 4 months, but thereafter the rate decreased sharply (text fig. 8).

A length of 24 to 30 mm. was reached at the age of 6 months and 36 to 40 mm. at the end of the first year. Individuals in nature may exceed 50 mm. in length at an estimated age of 2 years, with an ultimate length of 60 mm. occasionally. On the coast of Southern California growth and reproduction continue throughout the year.

C. fornicata

On the southern coast of New England the rates of growth of individuals of this species during the summer agree closely with those mentioned for C. onyx. With the advent of lower temperatures the rate decreases, to be increased again the following spring. Most of the young remain in the male phase during their first winter and transform to the female phase

during the following summer. They are then about 1 year old and 18 to 28 mm. in length. Isolated individuals, however, may complete a brief male phase, transform to females and reach a length of 28 mm. when 6 months of age if hatched in May.

C. nummaria

The common, dwarf, form is most often found in shells which limit the growth to a length of about 10 to 16 mm. (pl. 2, fig. 16a; text fig. 4, a). Those growing on unrestricted surfaces, however, may become 40 mm. or more in length, with a corresponding increase in width (pl. 2, figs. 17-27; text fig. 4).

When the young of the narrow dwarf form were allowed to attach themselves to the experimental bottles mentioned above, they quickly assumed the broad growth form (pl. 2, figs. 17-21, 23, 24, 27). The largest of these reached a length of about 40 mm. and a width of 25 mm. during a period of 5 months. The volume was then more than twenty times that of either of the parents. No further proof is needed that the dwarf form has the same inherent tendencies as the broad large form. During the period of sexual transformation an increment of 10 mm. in length during 1 month was recorded. Growth and reproduction continue throughout the year on the coast of Southern California.

C. plana

This closely similar species on the Atlantic coast has a correspondingly rapid growth during the summer months. An isolated male may complete the sexual transformation and reach a length of 25 mm. when 7 months old if hatched in early summer. More frequently, however, the male phase, with a length of 6 to 14 mm. is retained until the following spring or, if suitably mated, throughout its second summer or even longer (Coe, '36).

C. lingulata

The male phase becomes functional when the individual is only 2 to 3 weeks old and the shell only 4 to 6 mm. in length.

The sexual transformation usually occurs when the shell is 9 to 11 mm. long but earlier if isolated. The mature female is normally only 15 to 18 mm. in length when 5 months old and grows but little thereafter.

C. convexa and C. adunca

In both these species growth is comparatively slow. In both, the male phase becomes functional a few weeks after hatching. The shell is then only 3 to 6 mm. long. The sexual transformation takes place about a month later if isolated but when suitably mated the male phase may be retained for several months (Coe, '38a). The ultimate length of the shell seldom exceeds 10 to 16 mm. in C. convexa and 14 to 18 mm. in C. adunca. These dimensions may be reached at the age of about 6 months.

DISTURBANCE RINGS; GROWTH RINGS

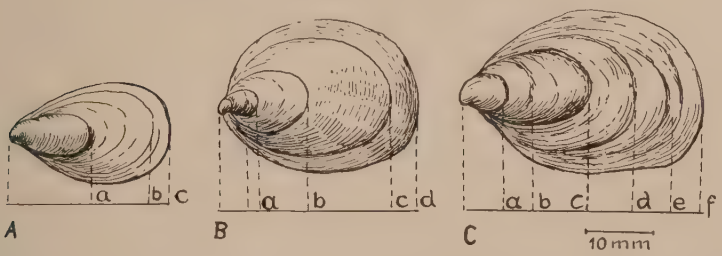

Fig. 8 C. onyx; Transformation of narrow growth form to broad growth form (rugosa) by transferring young male of length indicated by *a* to flat surface; increase in length from *a* to *b* required 2 months (including transformation of sex) and from *b* to *c* 1 month (including ovulation and incubation of young). *B*, increase from *a* to *b* (male phase), 2 months; *b* to *c* (transition and early female phases), 2 months; *c* to *d*, 1 month. *C*, increase from *a* to *b* (male phase), 1 month; *b* to *c* (transition), 1 month; *c* to *d*, *d* to *e*, and *e* to *f* (female phase), each 1 month. Natural size.

Whenever the young Crepidula moves to a new situation or is disturbed sufficiently to cause the withdrawal of the mantle from the edge of the shell, a record is usually made on the shell in the form of a "growth ring". The new shell later deposited does not conform exactly with the edges of the old shell, since

the new shell underlies the old and raises it slightly. The greater the disturbance the more conspicuous is the ring. The termination of the male phase is often similarly recorded on the shell because the slow, intermittent growth of the mated male is followed by the rapid secretion of smooth new shell during the period of sexual transformation. Such disturbance rings are clearly shown in text figure 8 and in plates 1 and 2.

SUMMARY

1. The rates of growth in seven species of the genus Crepidula were found to vary with the species, with the age, with the season and with environmental changes under experimental conditions. In the most rapidly growing species an increment in length of 4 to 10 mm. in 1 month was recorded.

2. In all the species the growth rate of isolated male-phase individuals was higher than that of mated males.

3. The most rapid increase in size occurs during the period of transformation from male to female phase; at that time the cytolysis and assimulation of all the spermatogenic tissues is in progress.

4. All except one of the species have widely deviating growth forms in nature, with dwarf and large individuals, differing in shape, color and texture of shell. Young of the dwarf form can be changed experimentally into individuals six to twenty or more times the size of either of their parents. The shape and size of the shell can be controlled by changing the shape and size of the object to which the foot is attached.

5. The sexual conditions likewise respond to environmental agencies to the extent that the male phase can be aborted, accentuated or prolonged and the functional female phase inhibited, but the sequence of the male and female phase cannot be reversed.

6. Individuals of some of the species will live for several months to a year in the running water of the aquarium, although little, if any, growth occurs without artificial feeding. When such stunted animals are transferred to the sea, normal growth and reproduction are resumed.

LITERATURE CITED

Coe, W. R. 1936 Sexual phases in Crepidula. J. Exp. Zool., vol. 72, pp. 455–477.

———— 1938a Conditions influencing change of sex in mollusks of the genus Crepidula. J. Exp. Zool., vol. 77, pp. 401–424.

———— 1938b Influence of association on the sexual phases of gastropods having protandric consecutive sexuality. Biol. Bull., vol. 75, pp. 274–285.

Conklin, E. G. 1897 The embryology of Crepidula. J. Morph., vol. 13, pp. 1–226.

———— 1898 Environmental and sexual dimorphism in Crepidula. Proc. Acad. Nat. Sci., Philadelphia, pp. 435–444.

Gould, Harley N. 1917 Studies on sex in the hermaphrodite mollusc Crepidula plana. I. History of the sexual cycle. J. Exp. Zool., vol. 23, pp. 1–69.

Ishiki, Hitoshi 1936 Sex changes in the Japanese slipper limpets, Crepidula aculeata and Crepidula walshi. J. Sci. Hiroshima Univ., Ser. B, Div. 1, vol. 4, pp. 91–99.

Orton, J. H. 1928 Observations on Patella vulgata. Part 2, Rate of growth of shell. J. Mar. Biol. Ass., vol. 15, pp. 863-874.

Pelseneer, Paul 1935 Essai d'éthologie zoologique d'apres l'étude des mollusques. Pub. Fond. Agathon De Potter, No. 1, Acad. Roy. Belgique, pp. 1–662.

Russell, E. S., 1909 The growth of the limpet shell of Patella vulgata L. Proc. Zool. Soc. London, 1909, pp. 235–253.

PLATE 1

EXPLANATION OF FIGURES

1 to 15 Crepidula onyx grown under natural and experimental conditions. Natural size.

1 Growth form onyx, male phase, detached from group of larger individuals; ages 1 to 3 months.

2 Growth form rugosa, male phase; ages 2 to 4 months.

3 Growth form onyx experimentally changed to rugosa by isolation of young male upon a flat surface.

4 Growth form rugosa; female phase with attached male.

5 Growth form onyx in female phase; maximum size on substrate of limited area. Compare figure 15.

6 Same with attached male.

7 Growth form onyx 1 month after attachment to flat surface. Point of arrow indicates size at beginning of experiment.

8 to 12 Initial growth form onyx transformed to rugosa after attachment to flat surface for 2 to 3 months, showing abrupt change in shape of shell with concurrent change of sexual phase. Points of arrows indicate sizes at beginning of experiment.

13 Female rugosa 6 months after attachment of male onyx to flat surface. Point of arrow indicates size at beginning of experiment.

14 Growth form rugosa; female phase; age about 1 year.

15 Growth form rugosa; estimated age 2 years.

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PLATE 2

EXPLANATION OF FIGURES

16 to 27 Crepidula nummaria grown under natural and experimental conditions. Natural size.

16 Slender dwarf form from inside shell of Tegula; male phase; ages 2 weeks to 2 months.

16a Slender form; dwarf females; largest size possible in shell of Tegula; age about 6 months; two individuals have mated males attached. Compare figure 27, showing individual of same age grown experimentally on substrate allowing unlimited space for growth.

17 to 21 Broad form; 2 to 3 months after transfer of the young of dwarf form to the experimental jar. All in female phase; four individuals with males in mating position.

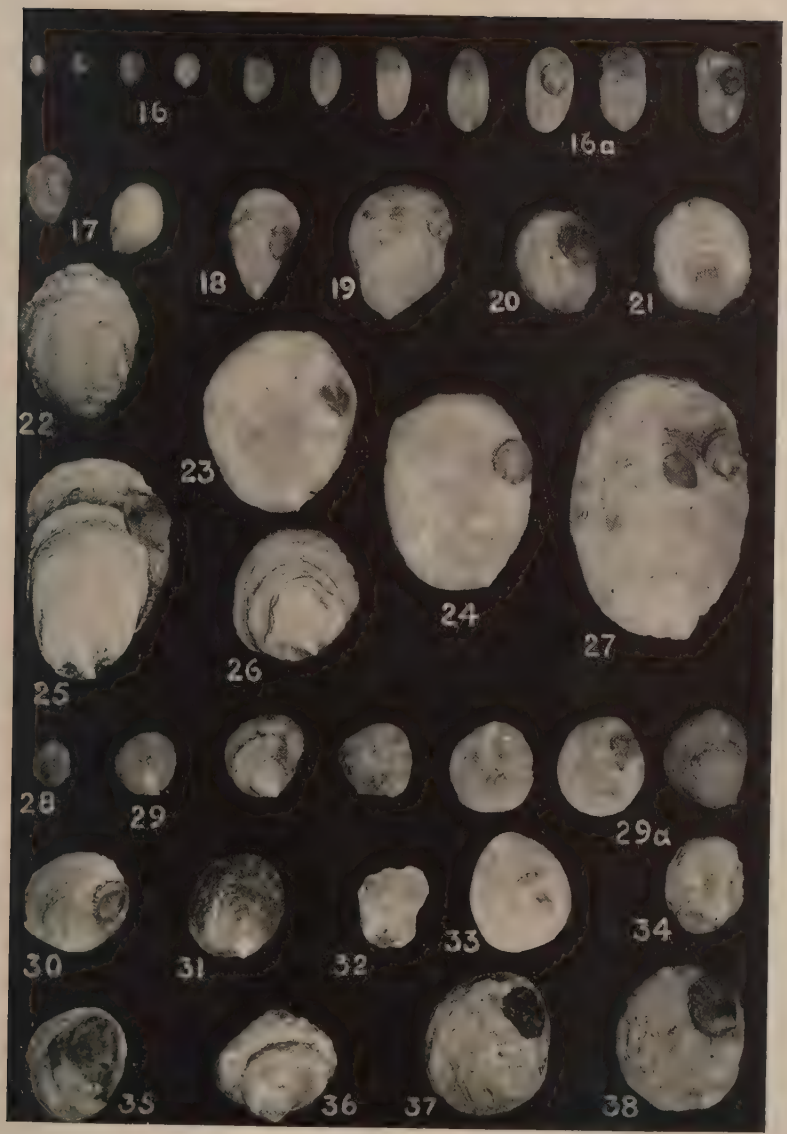
22 Broad form, grown on exposed rock.

23, 24 Broad form about 5 months after transfer of young male to experimental jar; both have mated males attached.

25, 26 Broad form; old, thick-shelled females from inside shell of large snail.

27 Broad form. Large female with two attached males; 5 months after transfer of young male of dwarf form to experimental box. Compare figure 16a, showing dwarf females of same age with growth limited by small size of substrate.

28 to 38 C. lingulata. Variations in form of shell when grown under different environmental conditions. All except figure 28 in female phase; mated males in figures 29a, 30, 35, 37, 38. Shell is high and subconical when grown on restricted surface; flat and irregular when beneath a rock; or circular and smoothly rounded when grown in quiet water on an unrestricted surface, as in the experimental jars.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Contribution No. 163

Reprinted from SCIENCE, May 8, 1942, Vol. 95,
No. 2471, pages 478–479.

SOME OBSERVATIONS ON THE FEEDING HABITS OF THE OCTOPUS[1]

IN recent years much has been written pertaining to the feeding habits of marine animals and of the minute details of the mechanisms involved in the capture and ingestion of their food. These studies result from an appreciation of the fact that the fundamental relationship among different species of a community of animals is a nutritional one, and in order to better understand the ecological implications, it is essential to know how the animals as individuals are able to capture their food or to escape the enemies preying upon them. The type of food utilized in any specific case is determined (sometimes within narrow limits) by the type of feeding mechanism possessed by the organism. Animals capable of active locomotion must seek out biotopes which support their food and the density of the organisms preyed upon will be determined in large measure by the efficiency of the catching apparatus of the predator, which is thus a factor in the balance of nature.

A common method of capturing food is by means of netting or screening devices such as the fine bristles on the appendages of barnacles, copepods, etc., or the gill rakers of fishes or whalebone of baleen whales. These netting devices, owing to their sieve-like nature, function to *concentrate* finely divided and scattered planktonic food gathered from a large quantity of water. A study of the feeding habits of the octopus reveals an ensnaring device of quite different nature, since, though it functions to make a multiple catch, it is not designed to filter but rather to impound.

The commonly observed method of gathering food by the octopus is that of lying in wait or stealthily approaching and suddenly striking out at its prey with a tentacle and grasping it with the aid of the

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 163.

suckers. But in addition to this method, small prey such as shrimp and small fish which live among low-growing seaweeds on the bottom, and which possess great agility in escaping the predator, may be caught in larger numbers by throwing over them a canopy-like web formed by the loose extensible membranes of skin connecting the bases of the tentacles and extending for some distance along the sides of each. This method of feeding was observed in the field on several occasions for large specimens of *Paroctopus apollyon* near the University of Washington Oceanographic Laboratories at Friday Harbor, Washington, and in view of the apparent want of published records of similar observations, it is deemed worth a brief note at this time.

The fishing action consists essentially of four steps: (1) gliding stealthily forward over seaweed beds while three or more tentacles are extended forward and high off the bottom; (2) the upraised tentacles are then slowly arched, bringing the distal ends downward with almost imperceptible movement to the bottom where the end suckers presumably attach to the hard bottom; (3) when this has been accomplished the loose web of the tentacles is extended with incredible speed so that the membranes of skin between adjacent tentacles form a closed canopy or "net" under which the shrimp and fish lurking in the sea weeds are impounded; (4) now the canopy is gradually diminished in size by pressing the tentacles closer to the ground in such a manner that the catch is slowly forced inward towards the mouth. The whole process is then repeated, sometimes in quite rapid succession. One specimen kept under observation from a rowboat made fifteen "hauls" in twenty minutes. The method is sometimes modified somewhat to a more commonly observed one whereby the animal pounces forward upon the prey with a less extended canopy.

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Contribution No. 166

CONCERNING THE HITHERTO UNKNOWN MALES OF THE COPEPODS *MICROSETELLA ROSEA* (DANA), *VETTORIA GRANULOSA* (GIESBRECHT), AND *CORISSA PARVA* FARRAN

MARTIN W. JOHNSON

Reprinted from TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY
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CONCERNING THE HITHERTO UNKNOWN MALES OF THE COPEPODS *MICROSETELLA ROSEA* (DANA), *VETTORIA GRANULOSA* (GIESBRECHT), AND *CORISSA PARVA* FARRAN[1]

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There is frequently a great inequality in the numbers of individuals of the sexes of planktonic copepods, usually the males being the fewer. The underlying causes of these differences are not fully known and are doubtless not the same for all species. The differences may appear to be permanent features of the population or they may be seasonal phenomena as indicated for the much studied *Calanus finmarchicus* for example. A seeming inequality may also result from a difference in the habits of the two sexes which tends to segregate them into more or less isolated groups. This was shown in the investigations of Nicholls (1933) dealing with diurnal reactions of *Calanus finmarchicus*. In this species the males, at the season of sampling, showed a lesser inclination to migrate toward the surface at night than did the females, thus effecting a separation showing a disproportionately large number of females if only the surface water strata is sampled. The segregation may become more complete and permanent if there is a difference in rate or direction of flow of the water strata inhabited by the sexes during the period of vertical segregation.

Frequently the sexes are wholly or practically indistinguishable before the fifth or sixth copepodid stages, the sixth being the adult stage. This coupled with an apparently ephemeral adult male stage of some species (Johnson 1938) must result in their less frequent capture in the plankton.

The life histories of the species here dealt with are unknown but in view of the great length of time (90 years) that the female of *Microsetella rosea* for example has been known, strongly suggests that the males of at least that species are actually relatively few at all times or that the recognizable male stages are of brief duration. The species is widely distributed especially in southern seas, occurring in the Mediterranean, Red Sea, Atlantic, Indian and Pacific Oceans. The concentration of individuals is not great but the frequent occurrences make the species a part of many reports dealing with the copepoda.

[1] Contributions from the Scripps Institution of Oceanography. New Series, No. 166.

EXPLANATION OF PLATE I

FIG. 1. Lateral aspect.
FIG. 2. Fifth pair of feet.
FIG. 3. First foot.
FIG. 4. Posterior part of body, dorsal.
FIG. 5. First antenna.

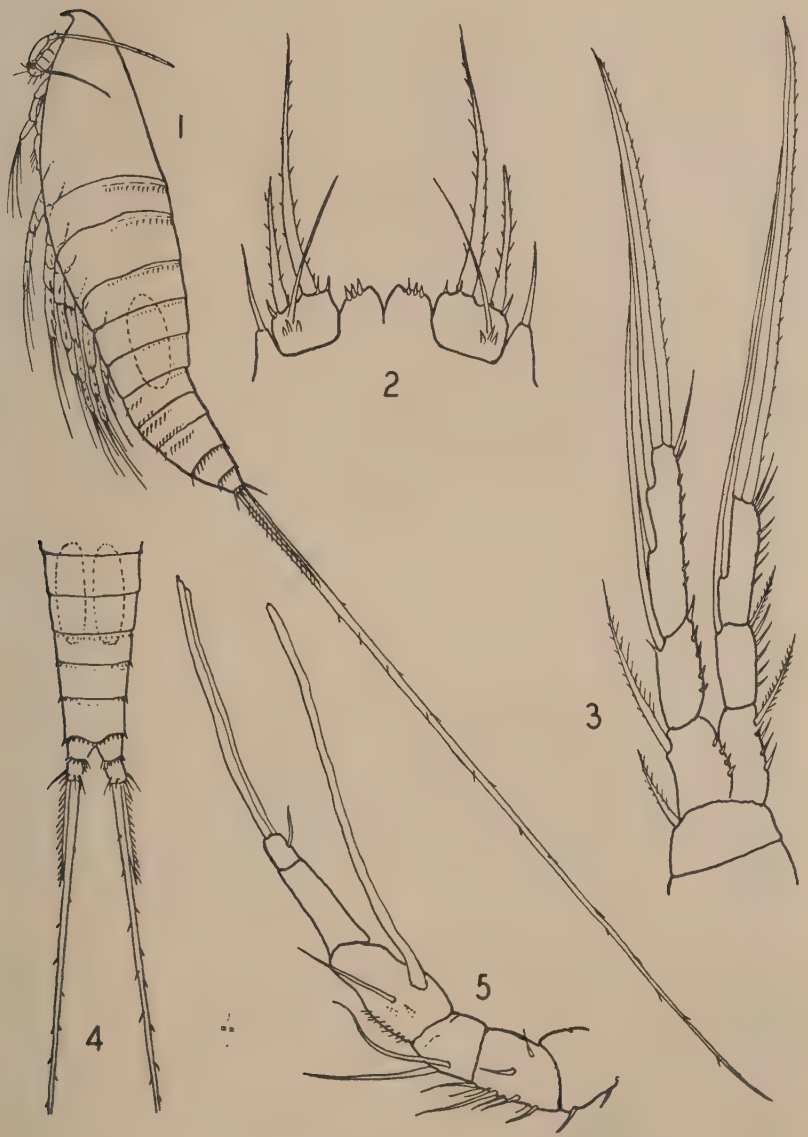

PLATE I. *Microsetella rosea*

Two other species here included are less common and more restricted in distribution, seeming to thrive best in offshore deep waters of lower latitudes.

Microsetella rosea (Dana)
Syn. *Canthocamptus roseus* Dana 1852

Male. Length 0.6 mm. to 0.7 mm. The body (Plate I, Figs. 1 and 4) is cylindrical, tapering at both ends as in the female, and the rostrum is sharply deflected ventrally. The urosome is as broad as the metasome and consists of five segments. The genital segment is divided, providing the most obvious characteristic separating the male from the female in which this segment is undivided. A more striking mark of distinction is, however, the conspicuous spermatophores showing through the body of the ripe males. The caudal rami are nearly as wide as long and may be somewhat divergent Each ramus bears one long coarsely setose spine nearly one and two-thirds the length of the body. Close at the base of each long spine is an outer shorter plumose spine.

The first antennae (Plate I, Fig. 5) are composed of six segments and are geniculate between the fourth and fifth segments. A long aesthetask is borne on the fourth and terminal segments.

The first four pairs of feet are provided with three segmented rami as in Plate I, Fig. 3; the fifth pair are trilobate (Plate I, Fig. 2) and the inner expansion of the basal segments of each leg is fused with its fellow on the opposite side and bears three short spines of somewhat varying length. The outer expansion bears a single smooth seta, while the terminal segment constituting the middle lobe, bears two strong setose terminal setae the inner of which is about twice the length of the outer. A smooth seta occurs on the anterior surface of the segment. These appendages differ from the corresponding male appendages of the closely related *M. norvegica* mainly in the greater strength of the shorter terminal spine which in *M. norvegica* is weak and smooth (Geisbrecht 1892, Plate 44). However, the great difference in body size and in relative length of the caudal spines are the chief characteristics separating the two species.

Occurrence. Microsetella rosea is found all along the west coast of the United States. The specimens upon which the present report is based were obtained in 200–0 meter hauls off San Diego in May, and San Francisco in June.

EXPLANATION OF PLATE II

FIG. 6. Dorsal aspect.
FIG. 7. Posterior division of body, lateral.
FIG. 8. First antenna.
FIG. 9. Mandible—enlarged scale.
FIG. 10. First maxilla—enlarged scale.
FIG. 11. Second antenna.
FIG. 12. Second maxilla—enlarged scale.
FIG. 13. Maxilliped.
FIG. 14. First foot.
FIG. 15. Second foot.
FIG. 16. Third foot.
FIG. 17. Fourth foot.

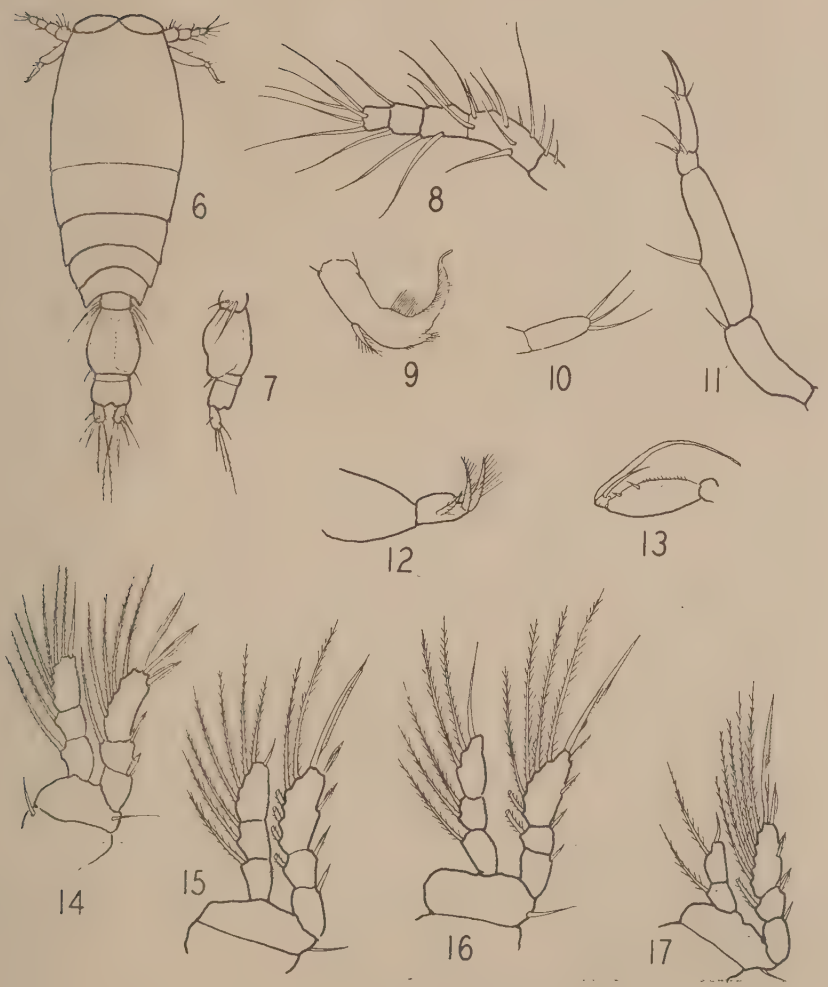

PLATE II. *Vettoria granulosa*

Vettoria granulosa (Giesbrecht)

Syn. *Corina granulosa* Giesbrecht 1891. (See Wilson 1924)

Male. Length 0.58 to 0.6 mm.

In general appearance the body (Plate II, Figs. 6 and 7) is as in the female with similarly granulated texture over the whole body, contingent cuticular lenses, and a very narrow fifth thoracic segment. The genital segment is long and broad having its greatest width just posterior to the middle and is provided on each posterior-lateral margin with a slender seta. Between the genital and anal segment there is present a short segment which in the female is wanting. The caudal rami are short as in the female, being about equal in length to the anal segment. The genital segment is about as long as the other urosome segments combined including the caudal rami.

The first antennae (Plate II, Fig. 8) are as in the female with five segments. The uniramous second antennae (Plate II, Fig. 11) are longer than the first antennae, and are each composed of four segments and a terminal stout claw; the proportional lengths of the segments one to four are: 2.5; 3.7; 0.5; 1.3.

The mandible (Plate II, Fig. 9) is composed of a claw-like structure with a narrow palm bearing a group of fine hair-like setae.

Each first maxilla (Plate II, Fig. 10) is a short process with three terminal setae.

Figure 12 (Plate II) shows the second maxilla which is composed of two segments, the distal one armed with three strong claws.

The maxilliped (Plate II, Fig. 13) consists of a very short basal segment followed by a long broad segment bearing two small spines on the distal half. The very long curved terminal claw reaches beyond the proximal end of the basal segment.

The first three pairs of swimming feet have three-segmented rami, the fourth pair have only two segments in the endopod (Plate II, Figs. 14, 15, 16 and 17).

The fifth pair of feet are reduced to one long and two shorter setae on each side of the fifth thoracic segment.

Occurrence. Several males together with females were taken in 200–0 meter hauls by Dr. E. G. Moberg aboard the U. S. cruiser *Louisville* at

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EXPLANATION OF PLATE III

FIG. 18. Dorsal aspect.
FIG. 19. Posterior division of body, lateral.
FIG. 20. Mandible—enlarged scale.
FIG. 21. First antenna.
FIG. 22. First maxilla—enlarged scale.
FIG. 23. Second antenna.
FIG. 24. Second maxilla—enlarged scale.
FIG. 25. Maxilliped.
FIG. 26. Fourth foot.
FIG. 27. Third foot.
FIG. 28. Second foot.
FIG. 29. First foot.

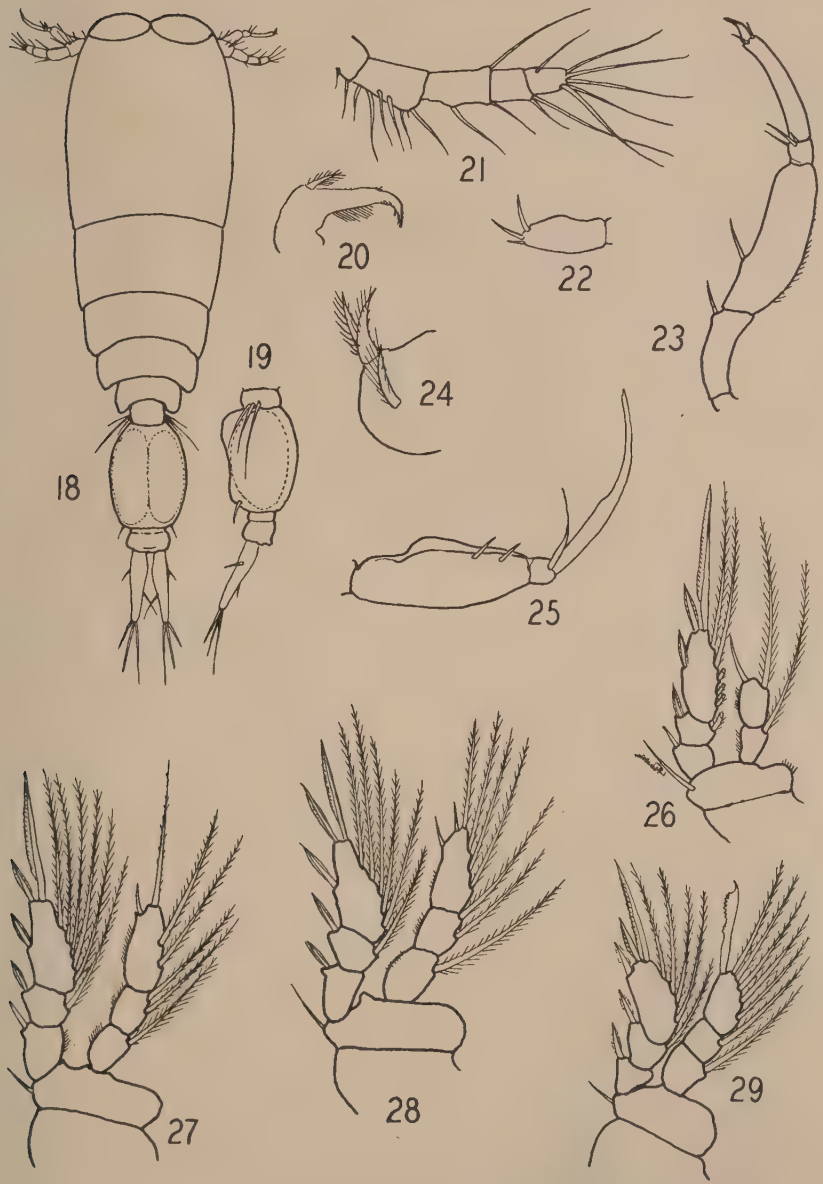

PLATE III. *Corissa parva*

oceanographic stations occupied midway between the California coast and the Hawaiian Islands at lat. 27°50′, long. 135°25′ and at lat. 26°13′, long. 142°02′. In the Pacific the species has been previously reported only from near the Galapagos Islands.

Corissa parva Farran (1936)

Male. Length 0.88 to 1.0 mm.

In general appearance the anterior division of the body (Plate III, Figs. 18 and 19) closely resembles the female and also the foregoing species. The fifth thoracic segment is very narrow, the cuticular lenses are contingent and occupy the whole front of the head, the body surface is finely granulated. The genital segment, unlike the male of *Vettoria*, is symmetrically oval in dorsal aspect having its widest point at the middle and bears on each side a small posterior-lateral seta. A very short narrow segment occurs between the genital and anal segments. The anal segment is tumid and broader than long. The caudal rami are long and narrow being slightly over three times longer than their greatest width.

A yellow pigmentation occurs on the genital segment and around the cuticular lenses and to a lesser extent also on the thoracic segments and maxillipeds.

The appendages are nearly as in the foregoing species except for certain significant differences as noted below for the second antenna, maxillipeds and swimming feet.

Proportionate lengths of the four segments of the second antennae are: 2.1; 3.9; 0.6; 2.8 (Plate III, Fig. 23).

The terminal claw of the maxilliped reaches only to about the basal segment (Plate III, Fig. 25).

The terminal spine of the first endopod is markedly modified as shown in Plate III, Fig. 29. The end segment of the second and of the third endopod each bear one more short smooth setae on the distal outer margin (Plate III Figs. 27 and 28) than is found in *V. granulosa.*

Occurrence. Several males and females at the same *Louisville* stations as mentioned above. Previously the species has been reported only from the region of the Great Barrier Reef.

Summary

In many species of planktonic copepods there is a great inequality in the numbers of individuals of the two sexes, usually the males are the fewer. This difference may be real or only apparent and may result from: seasonal fluctuations in the male population; difference in migratory habits which tend to segregate the sexes; and morphological obscurity of the males during submature stages coupled with an ephemeral recognizable adult stage.

The hitherto unknown males of the following species are here described in detail: *Microsetella rosea* (Dana), *Vettoria granulosa* (Giesbrecht), and *Corissa parva* Farran.

Bibliography

Farran, G. P. 1936. Copepoda. British Museum, Great Barrier Reef Expedition 1928–29. Scientific Report, 5 (3): 73–142.

Giesbrecht, W. 1892. Systematik und Faunistik der Pelagischen Copepoden des Golfes von Neapel. Fauna und Flora des Golfes von Neapel. 19: 831.

Johnson, M. W. 1938. Concerning the copepod *Eucalanus elongatus* Dana and its varieties in the Northeast Pacific. Bull. Scripps Inst. Oceanog. Tech. Ser. 4 (6): 165–180.

Nicholls, A. G. 1933. On the biology of *Calanus finmanchicus*. III. Vertical distribution and diurnal migrations in the Clyde Sea-Area. Jour. Mar. Biol. Assoc. 19 (1): 139–164.

Wilson, C. B. 1924. New North American parasitic copepods, new hosts, and notes on copepod nomenclature. Proceedings, U. S. Nat. Museum. 64: 1–22.

Contribution No. 167

Reprinted from CHRONICA BOTANICA
Vol. VII, 5, pp. 196-198, September, 1942

DENIS L. FOX: **The Carotenoid Pigments of Cryptogams***:- The carotenoid pigments of cryptogamic plants, notably among the algae, fungi and bacteria, have received critical attention only within late years. Studies of the lipochromes of algae are given special impetus by recent and current investigations by HEILBRON and his associates. They isolated a new carotenoid, myxoxanthin, from the blue-green alga *Rivularia nitida* (HEILBRON, I. M., B. LYTHGOE, and R. F. PHIPERS, Nature 136:989, 1935); E. LEDERER (Bull. Soc. Chim. Biol. 20:567, 1938) has pointed out chemical and physical resemblances between myxoxanthin and echinenone, the latter isolated by him from the female gonads of sea-urchins. Both pigments are vitamin A precursors with ketonic properties, similar chromatogams and similar absorption spectra. HEILBRON and PHIPERS (Biochem. Jour. 29:1369, 1935) have given special attention to fucoxanthin, a xanthophyll which occurs characteristically in brown algae. They believe that zeaxanthin may be a post mortem product of fucoxanthin, but numerous investigators regard this conclusion with considerable doubt, especially in view of the fact that whereas the tendency of carotenoids in decomposing tissue is to undergo oxidation instead of reduction, fucoxanthin contains six atoms of oxygen while zeaxanthin contains only two. HEILBRON, PARRY and PHIPERS (Biochem. Jour. 29:1376, 1935) have studied biochemical differences, including differences in recovered carotenoids, between the families of algae and between these and higher plants.

CARTER, HEILBRON and LYTHGOE (Proc. Roy. Soc., B. 128:82, 1940) have conducted an investigation of the lipochromes and sterols of representative members of seven algal classes. They find that the *Chlorophyceae* resemble higher plants in synthesizing carotene and lutein, with a few exceptions; characteristic of the *Phaeophyceae* is their synthesis of fucoxanthin, carotene and fucosterol. *Bacillariophyceae* and *Chrysophyceae* show close relationships to the more primitive orders of *Phaeophyceae*. The *Rhodophyceae* contain carotene and lutein, and show in this regard no relationship with the *Myxophyceae*, all of which contain the unusual carotenoid myxoxanthin. The authors believe that the absence of sterols in the *Myxophyceae* may be correlated with lack of sexuality in this class. Continued studies along the lines initiated by HEILBRON and his colleagues should be informative regarding relative evolutionary positions of the algal families themselves, the achlorophyllic plants lower in the scale, and the flagellate protozoa just above.

J. TISCHER (Z. Physiol. Chem. 1936, 1937, 1938, 1939) has written a series of seven papers on the carotenoids of fresh-water algae. A new xanthophyll, euglenarhodon, was found in the flagellate *Euglena heliorubescens*. Another flagellate, *Haematococcus pluvialis*, also contained euglenarhodon as the chief carotenoid, esterified with palmitic and other acids, and yielded in addition another new xanthophyll, hematoxanthin. Three new polyenes were encountered in the blue alga *Aphanizomenon flosaquae*, namely, aphanin, flavacin and aphanizophyll.

PACE (J. Biol. Chem. 140:483, 1941) reports finding in the diatom *Nitzschia closterium* chlorophyll *a* and chlorophyll *b* in the ratio of about 14 to 1, this proportion showing agreement with that found for *Phaeophyceae* generally, and being in contrast with the usual ratio of about 2.5 to 1 reported for *Chlorophyceae* and higher green aquatic plants. PACE measured relative amounts of xanthophylls and carotenes, and found the total quantities of each class to be present in the ratio of about 8.35 to 1. He found and measured the quantities of beta-carotene, cryptoxanthin, lutein, isolutein, zeaxanthin, and violaxanthin. PACE emphasizes the value of utilizing pigment analyses in estimations of phytoplankton populations.

GILLAM, EL REDI and WIMPENNY (J. Exp. Biol. 16:71, 1939) used quantitative determinations of carotenoids, chlorophyll and vitamin A in studying the seasonal variation in biological composition of plankton samples from the North Sea.

CHODAT and HAAG (Compt. rend. Soc. Phys. et Hist. nat. Genève 57:265, 1940) have made some studies relative to the concomitant accumulation of carotenoids and lipoids in green algae.

The LWOFFS (M. and A. LWOFF, Compt. rend. Soc. biol. 105:454, 1930) described the unique and specific role of acetate which led to massive carotenoid synthesis in light, by the flagellate *Haematococcus pluvialis*. They pointed out also that flagellates without chlorophyll, such as *Polytoma uvella*, *Astasia* spp., and *Euglena gracilis*, grown in the dark, synthesized carotene.

FOX and SARGENT (Chem. and Ind. 57: 1111, 1938) determined that the halophylic algal flagellate *Dunaliella salina* produced great quantities of beta-carotene and practically no chlorophyll if cultured in 25 per cent (fully saturated) sodium chloride solutions, whereas if grown in solutions of half to two-thirds this concentration, the cells were brilliant green instead of brick-red, showing high chlorophyll content and far less beta-carotene. Although the green variant grows more rapidly, both it and the red form show maximum positive phototaxis at wave-lengths between 480 and 530 mμ this region including the two chief absorption maxima of beta-carotene. (H. F. BLUM and D. L. FOX, Univ. of Calif. Publ. in Physiol. 8:21, 1933).

Studying carotenoid-sensitized photosynthesis in the diatom *Nitzschia closterium*, DUTTON and MANNING (Am. Jour. of Bot-

*Contributions from the Scripps Institution of Oceanography, New Series, No. 167.

any 28:516, 1941) report that, at a wavelength of 4960 Å, photosynthesis proceeds at a lower rate than at 6650 Å, probably because 90% or more of the absorbed light at the former region is taken up by carotenoids rather than by chlorophyll. On the basis of the calculated efficiency of photosynthesis at 4960 Å, DUTTON and MANNING conclude that light absorbed by some or all of the carotenoids in this diatom is utilizable in photosynthesis.

LEDERER (Bull. Soc. Chim. Biol. 20:609, 1938) has reviewed his own work and that of others on the carotenoids of various bacteria and fungi. These cryptogams often yield unique pigments, sometimes in very complicated mixtures.

Systematic studies of carotenoids in bacteria, and of factors which lead to chromogenesis in these forms have been made within the last decade. CHARGAFF (Compt. rend. Acad. Sci. 197:946, 1932) and CHARGAFF and DIERYCK (Naturwiss. 20:872, 1932) investigated the carotenoids of *Sarcina lutea, Sarcina aurantiaca, Staphylococcus aureus* and *Mycobacterium phlei,* finding a number of familiar pigments and a new one which they called sarcinine.

BAUMANN, INGRAHAM, STEENBOCK and FRED (Jour. Biol. Chem. 103:339, 1933) studying the vitamin A potency of numerous colored bacteria, found that all the vitamin activity was accounted for by carotenoids, but that vitamin A as such was quite absent. Similar results were obtained by INGRAHAM and BAUMANN (Jour. Bact. 27: 25, 1934) in a study of carotene synthesis by bacteria. They also found that in *Mycobacterium phlei,* lipid storage and utilization were paralleled by respective pigment production and disappearance.

INGRAHAM, FRED and STEENBOCK (Wis. Agr. Exp. Sta., Bull. 430 (Ann. Rep. 1933-1934):104, 1935), studying factors which might affect pigmentation in bacteria, found that, while any of a number of carbon sources favored growth, the addition of glycerol to cultures resulted in a maximal production of pigment, including carotene. They reported that no anaerobes synthesized carotenoids.

INGRAHAM and STEENBOCK (Biochem. Jour. 29:2553, 1935) determined that *Mycobacterium phlei* did not exhibit increased pigmentation when cultured in the presence of any of a variety of organic acids, aldehydes, ketones or sugars, but that a few of the simple alcohols, and especially glycerol, greatly enhanced synthesis of carotenoids, while *p*H, light, temperature, ethylene, and a number of salts of sodium, lithium, calcium, magnesium or selenium had no specific effects on pigmentation.

Little is known of the physiological functions of carotenoids in bacteria. It is worth recalling that in the higher plants carotenoids are always accompanied by chlorophylls (excepting perhaps in a few parasitic forms), that carotenoids are not synthesized in animals (save in a few doubtful cases), and that bacteria, fungi and etiolated green plants are able to produce carotenoids in the absence of chlorophyll. Since in bacteria, carotenoids have been detected only in aerobic species, it is conceivable that the pigments may play a role in the physiological use of atmospheric oxygen. Again, the demonstrated utilization of glycerol in the synthesis of carotenoids by some bacteria suggests the possible association of these pigments with lipid metabolism. The ability of carotenoids to absorb light of shorter wave-lengths might also be of some physiological importance to bacteria containing such pigments.

Some workers believe that carotene-xanthophyll systems in certain microorganisms may play important roles in biological oxidation-reduction reactions. VAN NIEL and SMITH (Arch. f. Mikrobiol. 6: 219, 1935) found that the photosynthetic purple bacterium *Spirillum rubrum* showed strong phototactic response in those regions of the spectrum where the three absorption bands of the unique carotenoid spirilloxanthin occur, as well as in the regions where the green, presumably photosynthetic pigment shows absorption maxima.

Quite as spectacular and interesting as the carotenoid pigments displayed by certain bacteria are those encountered in numerous species of fungi, both macroscopic and microscopic. LEDERER (*op. cit.*) lists a number of unusual carotenoids, including some acidic pigments, found in species of Ascomycetes, Basidiomycetes and Myxomycetes, as well as in Schizomycetes examined by him and by others.

Investigations by the author (D. L. FOX, Proc. Nat. Acad. Sci. 23:295, 1937) have revealed the existence of considerable quantities of carotenoid pigments, accompanied by breakdown products of chlorophyll, in mud cores from the ocean floor. Further investigations in this laboratory (FOX and ANDERSON, Proc. Nat. Acad. Sci. 27:333, 1941) show a preponderance, in mud cores from the gulf of California, of epiphasic or carotene-like pigments characteristic of the achlorophyllic cryptogams, over the hypophasic or xanthophyllic pigments which predominate in algae. The fact would suggest that microörganisms such as fungi and bacteria may contribute more carotenoids to marine sediments than do falling diatoms and other algae. While it is not easy to conceive of active metabolism in fungi or bacteria which may be buried many feet in deep reducing marine muds, the concentration of carotenoids in mud layers 17 feet beneath the top (representing a residence of several thousand years) to the extent of 6 to 7 mg per 100 g (dry basis) at least suggests fragmentary biochemical chapters of past ages.

Carotenoid pigments play certain important physiological roles, sometimes in the presence of light, as in the visual cycle described by WALD, or in the sexual fusion of the isogametes of *Chlamydomonas eugametos.* The latter occurs through the participation of the carotenoid crocin (R. KUHN, F. MOEWUS and D. JERCHEL, Ber. Dtsch. Chem. Ges., 71B:1541, 1938; F. MOEWUS, Naturwissensch. 27:97, 1939). The molecular structure of carotenoids

which confers upon them the attributes of color and chemical reactivity may also constitute the basis of the importance of certain of them as vitamin A precursors, and in other biochemical processes including those which are associated with sexual reproduction.

Long before the important studies of MOEWUS and his colleagues, on the sexual role of crocin in the algal flagellate *Chlamydomonas*, others had observed carotenoid pigmentation in the reproductive cells of fungi. LENDNER (Bull. Soc. Bot. Genève 10:362, 1918) had noticed the pronounced yellow pigmentation in the large gametes of *Mucor hiemalis*. Likewise SATINA and BLAKESLEE (Proc. Nat. Acad. Sci., 12:191, 1926) observed the same sexual difference, accompanied by higher lipoid content in the (+) gametes of several *Mucoraceae*. CHODAT and SCHOPFER (Compt. rend. Soc. Phys. Hist. nat. Genève 44:176, 1927) extended studies of the relationship between sexuality and pigmentation by carotene(s) in *Mucor hiemalis*, expressing the belief that the higher carotenoid content of the (+) or female races reflects more intense metabolism and greater food storage than in the (—) or male plants. MURNEEK (Science 79:528, 1934) reported correlations between the carotenoid content of the leaves of higher plants and the reproductive cycle, carotenoids increasing steadily up to the time of flowering, then diminishing.

Work by EMERSON and FOX (Proc. Roy. Soc. (London) B 128:275, 1940) has shown a clear correlation between carotenoid pigmentation, sexuality and the reproductive cycle in the water mould *Allomyces*. The asexual phase is without carotenoids, while in the sexual phase the small male gametes show extraordinarily high concentrations of the rare pigment, gamma-carotene, limited exclusively to the male gametes and disappearing rapidly after their fusion with the larger colorless female gametes.

Prof. I. M. HEILBRON and Dr. E. R. H. JONES (Imperial College of Science and Technology, London; personal communication, 1939) have examined the pigments in the gametes of several species of brown marine algae, including *Ascophyllum nodosum, Fucus serratus* and some other species of *Fucus*. They have found beta-carotene preponderating in the male gametes, and fucoxanthin, accompanied by chlorophyll, in the female.

In view of the pronounced correlations which have been revealed between carotenoid pigmentation, sexuality and reproductive metabolism, in both plant and animal organisms, continued analyses and experimentation along these lines would seem very promising. Because of their greater simplicity, both in structure and in biochemical makeup, especially as regards relative numbers of carotenoids, the cryptogams present favorable material for continued studies of chemical, thermal and photic factors in chromogenesis and of possible physiological functions of carotenoids. Finally, those algae and fungi whose sex cells are differentiable are excellent forms for investigations of the biochemistry of sex and reproduction.

SCRIPPS INST. OF OCEANOGRAPHY OF THE U. OF CALIF., LA JOLLA, CALIF. *October 1941.*

Contribution No. 168

REPORTS FROM RECIPIENTS OF GRANTS FROM THE RESEARCH FUNDS

CARROLL WALTER GRANT, Brooklyn College

Grant No. 555 (1941), $500. Studies in marine microbiology at the Scripps Institution of Oceanography, University of California, La Jolla.

A general survey of the occurrence and importance of bacteria in the sea is being made with special reference to those exhibiting the distinctive features of agar liquefaction, photogenesis, chitin digestion, nitrogen fixation, hydrocarbon utilization and thigmotropism. A large number of bacteria which have been isolated from sea water and marine mud are being made the subject of investigation.

In collaboration with Dr. Claude E. ZoBell, studies have been made on the minimum nutritive requirements of marine bacteria. Sea water and added organic nutrients, ranging from one to one hundred milligrams per liter, have been used. Evidence has been produced in growth, total counts and oxygen utilization to show that quantities of nutrient material as low as that occurring either in natural sea water or only one milligram of glucose or peptone per liter support active bacterial multiplication. The rate of bacterial multiplication and respiration is more or less directly proportional to the concentration of organic matter. Concentrations of glucose ranging from one to five milligrams per liter are utilized quantitatively.

Several species of chitinoclastic bacteria have been isolated from sea water and marine sediments. Some of these exhibit striking coloration and marked thigmotropism. Their morphological, cultural and physiological characteristics are being investigated. The pigments produced by these organisms are being made the subject of special study in collaboration with Dr. Denis L. Fox, biochemist at the Scripps Institution of Oceanography.

Nonsymbiotic nitrogen-fixing bacteria have been isolated from sea water. Daily samples collected from the end of the Scripps Institution pier reveal the presence of 1 to 87 bacteria per cc. which multiply in a nitrogen-free synthetic sea water medium prepared with mannitol. This number may be contrasted with a heterotrophic bacterial population of 50 to 5,000 organisms per cc. which grow on a nutrient medium containing available nitrogen. Several bacteria are found in coastal waters which will grow in fresh water nitrogen-free mannitol medium. While it is possible

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that these organisms may be of terrestrial origin, it is significant that appreciable numbers of nitrogen-fixing bacteria have been found associated with kelp, considerable distances offshore. It is believed that the carbohydrate requirements of the nitrogen-fixing bacteria may be obtained from the disintegration of kelp. Some of the bacteria closely resemble the genus Azotobacter.

An effort is being made to isolate various kinds of bacteria capable of oxidizing different hydrocarbons. Such bacteria may play an important role in the accumulation of petroleum and may also influence the diagenesis of marine sediments.

Contribution No. 169

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pages 320–330.

BACTERIA OF THE MARINE WORLD[1]

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ALTHOUGH the ocean has been described by bacteriologists as the "world's largest and most efficient septic tank," there are some land-locked biologists who question the existence of bacteria and allied microorganisms in the sea beyond the littoral zones influenced by terrigenous contamination. Certain text-books published during the last decade state that it is virtually impossible for bacteria to thrive in the open ocean, due to a multiplicity of adverse environmental conditions. Purportedly these include the high salinity of sea water, the paucity of organic matter, the processes of sedimentation, the abundance of natural enemies, the germicidal effect of ultraviolet radiations near the surface and the high hydrostatic pressure and low temperature at greater depths.

Believing that high hydrostatic pressures are inimical to life, the author of a recent text-book on general physiology concludes that bacterial putrefaction does not occur at great depths, a deduction which is contradicted by the recovery of viable bacteria from great depths and by evidence of bacterial activity on the sea floor.

Most of the early surveys indicated that while bacteria could be demonstrated in sea water from certain localized regions near land, for the most part bacteria should be regarded as ephemeral transients from a more favorable terrestrial environment. More recent surveys[1a, 2] which have been made in this country and abroad show that bacteria are quite widely distributed in the sea and that they probably play an important role influencing chemical, geological and biological conditions.[3] Specialized analytical methods have revealed the presence of active bacteria wherever water or mud samples have been collected; in mid-ocean more than a thousand miles from land, in water at depths exceeding three miles and buried in fifteen feet or more of bottom sediments.

BACTERIA IN SEA WATER

Bacterial populations ranging from a few hundred to several thousand per cc of water are not uncommon, although there are extensive oceanic zones where there are fewer than one bacterium per cc. The distribution of bacteria is influenced by several interrelated factors of which the concentration of utilizable organic matter is the most important. Usually there are more bacteria along the littoral zones than farther at sea.

Neither latitude nor water temperature directly influence the distribution of bacteria in the sea because extensive populations have been found in Arctic and Antarctic as well as in tropical regions. Wherever other organisms have been found (either plant or animal), careful studies reveal the presence of bacteria and allied microorganisms. Indeed, bacteria have been demonstrated in many marine environments where there is no evidence of other organisms existing.

Water samples from different depths of the sea have been obtained for investigating the vertical distribution of bac-

[1] Contributions from the Scripps Institution of Oceanography, New series, No. 169.

[1a] S. A. Waksman, SCIENTIFIC MONTHLY, 38: 35–49, 1934.

[2] W. Benecke, Abderhalden's Handb. der biol. Arbeitsmethoden, Abt. 404, Lfg. 404: 717–872, 1933.

[3] S. A. Waksman, *Ecol. Monogr.*, 4: 523–529, 1934.

teria by means of a pressure-resistant collapsible rubber bottle stoppered with a hermetically sealed capillary glass tube. The apparatus can be sterilized by boiling or autoclaving and lowered into the sea clamped to a hydrographic wire or cable. When the desired depth is reached a messenger (a weight which slides down the cable) is dropped. The messenger strikes a lever which breaks the glass tube two or three inches from any source of extraneous contamination, thus permitting the sterilized bottle to fill with sea water.

Living bacteria have been recovered from a depth of 5,000 meters where the hydrostatic pressure is around 500 atmospheres, or 7,500 pounds per square inch. Although it is not known how many bacteria may be killed by being hauled rapidly to the surface from great depths, experiments indicate that most bacteria tolerate such changes in hydrostatic pressure.

Bacteria, like nearly all other forms of marine life, are more abundant in the topmost 100 meters of water than in any zone at greater depths. This is attributed to the greater abundance of food in the upper zone which is derived directly or indirectly from photosynthetic organisms. The vertical distribution of the latter is limited primarily by the depth to which sunlight penetrates. Water temperature and the concentration of plant nutrients are also important factors.

The concentration of plant nutrients is influenced by the vertical movement of water masses[4] as well as by the mineralizing activities of bacteria. Water from the photosynthetic zone at stations

[4] H. U. Sverdrup, *et al.*, "The Oceans: Their Physics, Chemistry and General Biology," 990 pp. New York: Prentice-Hall, Inc. 1942.

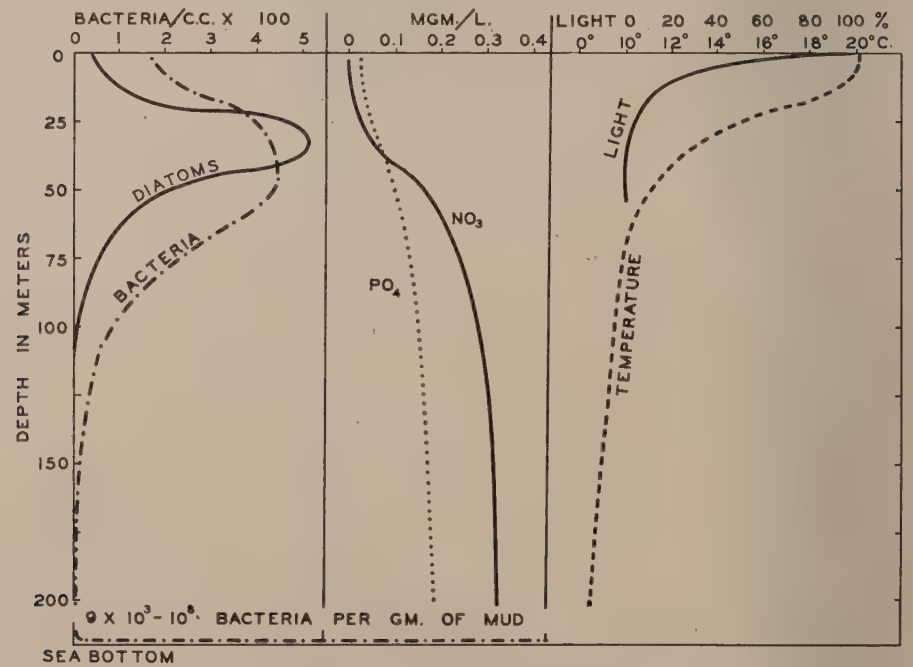


FIG. 1. VERTICAL DISTRIBUTION OF BACTERIA (NUMBER PER CC OF WATER), DIATOMS (NUMBER PER LITER OF WATER), PHOSPHATE, NITRATE, LIGHT AND TEMPERATURE IN THE SEA BASED UPON THE AVERAGE RESULTS AT SEVERAL DIFFERENT STATIONS OFF THE COAST OF SOUTHERN CALIFORNIA

remote from land generally contains from a few to a few hundred bacteria per cc. Occasionally several thousand bacteria per cc of water are found in certain localized areas.

At all latitudes where observations have been made the number of bacteria decreases rapidly with depth. This is illustrated by Fig. 1, which also shows the vertical distribution of phytoplankton, nitrate, phosphate, temperature and light. One rarely finds more than one to ten bacteria per cc below 200 meters except on the sea bottom.

The analysis of water samples collected daily from the end of the S.I.O. pier for a period of ten years reveals that the seasonal distribution of bacteria tends to follow that of phytoplankton. As a rule the largest bacterial populations occur from January to March and the smallest from May to July. However, localized conditions often cause marked fluctuations from day to day.

Bacteria in Marine Mud

The richest microflora is found on the bottom of the sea.[5] In spite of the fact that the temperature may be from $-0.5°$ to $+2.5°$ C. and the hydrostatic pressure may amount to several hundred atmospheres, or a few tons per square inch, there are often as many bacteria in marine bottom deposits as in fertile garden soil. The topmost layer of mud which is in contact with the overlying water usually contains several million viable bacteria per gram. The number is not a function of the latitude or of the depth of the overlying water, but it is related to the particle size of the sediment and the organic matter content. These properties of the bottom sediment are influenced by oceanic circulation, bottom topography, distance from land and several other other interrelated factors.[4]

The vertical distribution of bacteria

[5] C. E. ZoBell, *Jour. Sed. Petrol.*, 8: 10–18, 1938.

THE SCRIPPS INSTITUTION OF OCEANOGRAPHY

THE *E. W. Scripps*, WITH THREE SAILS UNFURLED, IS SHOWN APPROACHING THE END OF THE PIER WHICH EXTENDS 1,000 FEET OUT INTO THE SEA.

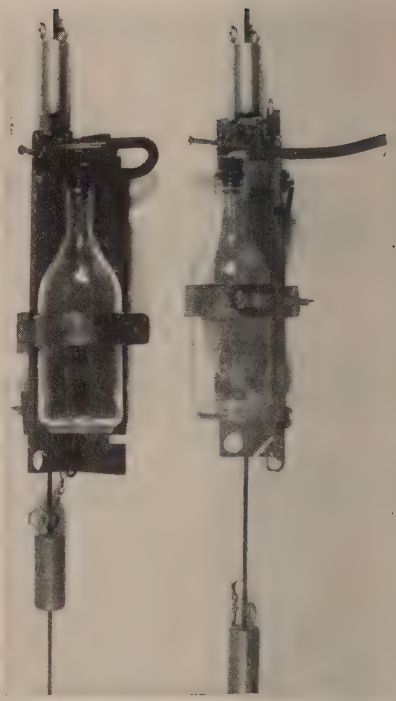
in the bottom deposits has been studied by analyzing long cylindrical cores of mud. The mud cores are collected in a celluloid-lined pipe. The pipe loaded with several hundred pounds of streamlined lead weights is forced into the bottom mud when it is dropped rapidly, held in a vertical position by the dredging cable. The corer is hauled upon the deck of the research vessel by a power winch and the celluloid barrel full of mud is removed. Radially central portions of the mud cores representing strata from different depths are dissected out aseptically and bacteriologically analyzed.

In the bottom mud the bacterial population decreases from the surface with depth. The bacteria are unevenly distributed, probably due to their tendency to colonize and due to chemical and physical differences within the mud itself. On the average the number of bacteria decreases more or less exponentially from several million per gram at the mud-water interface to a few thousand per gram at depths exceeding 25 cm. Below this depth the decrease is slow and gradual, hundreds of bacteria per gram having been found in mud cores exceeding 500 cm in length. Table 1 shows the number of bacteria which were found in a long mud core collected from the lower end of the Gulf of California at a station where the water was 2,230 meters deep.

TABLE 1

VERTICAL DISTRIBUTION OF AEROBIC AND ANAEROBIC BACTERIA IN A MUD CORE COLLECTED FROM THE GULF OF CALIFORNIA WHERE THE WATER WAS 2,230 METERS DEEP.

Core depth in cm.	Aerobes per gram	Anaerobes per gram
0– 10	62,000,000	8,900,000
10– 20	470,000	106,000
20– 30	235,000	73,000
40– 50	91,000	23,800
90–100	4,800	2,610
140–150	7,700	1,520
190–200	12,900	870
240–250	2,060	920
340–350	760	218
400–410	164	193
440–450	310	44
500–510	580	26



J–Z WATER SAMPLER

FOR BACTERIOLOGICAL WORK. THE WATER BOTTLE ON THE LEFT IS IN A POSITION TO COLLECT A SAMPLE OF SEA WATER. THE UPPER MESSENGER IS ABOUT TO STRIKE THE LEVER WHICH BREAKS THE CAPILLARY GLASS TUBE AT THE POINT INDICATED. WHEN THE TUBE BREAKS, THE PRESSURE RUBBER TUBING STRAIGHTENS OUT TO THE POSITION INDICATED AT THE RIGHT. SIMULTANEOUSLY THE LOWER MESSENGER IS RELEASED TO FALL ALONG THE HYDROGRAPHIC WIRE TO A SECOND WATER BOTTLE BELOW. GLASS BOTTLES AS ILLUSTRATED WITHSTAND HYDROSTATIC PRESSURE TO DEPTHS OF TWO OR THREE HUNDRED METERS; COLLAPSIBLE RUBBER BOTTLES ARE USED AT GREATER DEPTHS.

Aerobes as well as anaerobes occur in the mud. Although most of the bacteria have the faculty of living either in the presence or in the absence of free oxygen, some which appear to be strict aerobes are regularly found at the bottom of long mud cores where there is no free oxygen as manifested by the low oxidation-reduction potential and by the presence of hydrogen sulfide.

THE *E. W. SCRIPPS*
AN AUXILIARY SCHOONER, WITH RIGGING FOR UNFURLING 4,000 SQUARE FEET OF CANVAS, WHICH IS USED FOR STUDYING THE OCEAN.

The significance of the occurrence of strict aerobes in a highly reducing environment is a problem for speculation. Are they passive inhabitants of the anaerobic mud, preserved in a state of suspended animation since they were deposited from the oxygenated water several thousand years earlier, or do they respire and reproduce by a mechanism unknown to the microbiologist? Some of the secrets of life might be learned from the study of these unique marine microorganisms buried at the bottom of the sea.

Bacteria occur in the bottom deposits at depths far below that at which any other type of living organism is found. By consuming the oxygen from the interstitial water and otherwise rendering the environment reducing in character, they create conditions which are incompatible to the existence of other forms of life. The bacteria slowly decompose the organic matter which rains down from above, thereby effecting the chemical composition of the mud as well as altering its physico-chemical properties including the hydrogen-ion concentration and the oxidation-reduction potential.

Characteristics of Marine Bacteria

The bacteria which are isolated from the sea at places remote from possibilities of terrigenous contamination differ from their terrestrial cousins in many respects. Morphologically they are essentially like those which are indigenous to fresh-water or terrestrial environments with only minor points of difference.

Bacilli, or rod-shaped bacteria, constitute around 63 per cent. of the marine microflora, 5 per cent. are cocci or spherical and 32 per cent. are spirilla or helicoidal in shape. They average somewhat less than one micron in diameter. A large proportion of them are actively

motile by means of one or more flagella. Gram-negative forms predominate. Only a small percentage of them form endospores. Under suitable conditions somewhat more than half of the bacteria from the sea are chromogenic, producing yellow, orange, brown, red, purple, blue or sometimes green pigments.

Physiologically, marine bacteria are more distinctive, differing from terrestrial bacteria particularly in their salinity requirements. Most of the bacteria from the sea require sea water for their growth following initial isolation, while the bacteria from soil, sewage and other terrestrial sources grow poorly or not at all in sea water media. As shown by Table 2, only 8 to 17 per cent. of the

TABLE 2

Relative Numbers of Bacteria in Marine and Terrestrial Materials which Grew in Nutrient Media Prepared with Sea Water and Other Salt Solutions.

Sample	Number of samples analyzed	Salt solution used to prepare nutrient media			
		Natural sea water	Tap water	Synthetic sea water	3.0% NaCl
Sea water ..	12	100	8	78	56
Marine mud	12	100	17	73	64
Sewage	8	13	100	34	29
Tap water .	8	3	100	40	31
Soil	8	14	100	52	46

bacteria from sea water or marine mud grow in fresh-water media, while only 3 to 14 per cent. of those in sewage or soil grow in sea-water media. Neither synthetic sea water nor isotonic salt solution is an entirely satisfactory substitute for natural sea water.

Curiously enough, natural sea water is more toxic for fresh-water bacteria than is isotonic synthetic sea water. This is partly due to the presence in sea water of minute concentrations of thermolabile substances probably organic in nature which are bactericidal or bacteriostatic for fresh-water bacteria but do not injure marine forms.

Contrary to popular conception very few marine bacteria are halophilic or salt tolerant. Most of them require around 3 per cent. salt, but very few marine bacteria tolerate salt concentrations exceeding 6 per cent. Paradoxically a larger percentage of terrestrial than marine bacteria can grow in solutions containing from 6 to 10 per cent. salt. Korinek[6] and others[7] report that in general terrestrial or fresh-water bacteria tolerate changes in salinity and osmotic pressure better than marine bacteria.

The specific salt requirement of marine bacteria may account for the failure of early investigators to find appreciable numbers of bacteria in the sea except in bays, estuaries and littoral water laden with land contaminants. Being guided by conventional standard methods for the bacteriological analysis of water, the investigators prepared their media with fresh water or with ordinary salt.

Many present-day workers are investigating the bacterial flora responsible for the spoilage of catches of marine fish using fresh-water media. Upon a basis of such observations, they conclude that fresh-water or terrestrial bacteria are primarily responsible for the decomposition of marine fish. Other investigations indicate that there are many more proteolytic bacteria associated with marine fish which will grow on sea-water media than those which will grow on standard fresh-water media. This observation leads us to believe that if marine bacteria are treated as physiologically distinctive species, very different conclusions concerning the microflora of living and dead marine fish will be reached.

A large percentage of the bacteria found in the sea are actively proteolytic as indicated by their ability to liquefy gelatin or to decompose virtually all types of nitrogenous compounds with the liberation of ammonia. As a rule they

[6] J. Korinek, *Centralbl. f. Bakt.*, II Abt., 66: 500–505, 1926.

[7] C. E. ZoBell, *Jour. Mar. Res.*, 4: 42–75, 1941.

are less actively saccharolytic than freshwater or terrestrial bacteria in general, although among the hundred or so species of marine bacteria which have been studied are representatives which attack all the simple carbohydrates. Bacteria which digest starch, cellulose, chitin and lignin occur in the sea. Agar liquefiers are noted on nearly every plate inoculated with sea water or marine mud. Photogenic or luminescent bacteria are also common in the sea. Sometimes enough of them develop in sea water stored in the dark to make it glow with cold light.

Temperature Requirements

Since over 80 per cent. of the ocean is perpetually colder than 5° C., it is not surprising to find that many bacteria from such an environment are able to grow at relatively low temperatures. Although food refrigeration is founded on the principle that low temperatures inhibit bacterial activity, many marine bacteria grow well at refrigeration temperatures. Several species have been studied which multiply and are otherwise physiologically active at temperatures ranging from 0° to −11° C. Indeed, most marine bacteria multiply at 0° C. These cold-loving organisms are sometimes very troublesome in the spoilage of refrigerated foods, uncured furs and other products from the sea.

Marine bacteria as a group are much more thermosensitive than are most terrestrial bacteria. The ability of a group of pure cultures of marine bacteria to survive or to multiply at different temperatures is illustrated by the data in Table 3. Very few marine bacteria survive at temperatures which are optimum for the growth of many terrestrial bacteria, 30° to 37° C. Some marine bacteria are actually killed in 10 minutes at 30° C. Only about half of the marine bacteria studied by the author survived at 37° C. for ten minutes. However, as on the land there are a few spore-formers

TABLE 3

Number of Pure Cultures of Marine Bacteria which Survived at Different Temperatures for Ten Minutes and the Number which Multiplied in Nutrient Media at These Temperatures, 128 Being the Total Number Tested.

Temperature	Survived	Multiplied	Temperature	Survived	Multiplied
− 4° C.	128	103[a]	42° C.	51	8
+ 3° C.	128	128	45° C.	40	2
22° C.	128	128	50° C.	23	0
25° C.	128	112	60° C.	9	0
30° C.	123[b]	89	80° C.	7	0
37° C.	61	18	100° C.	2	0

[a] Evidence of multiplication after three weeks.
[b] Survived stated temperature for ten minutes.

in most samples of marine materials which are not killed by the boiling temperature of water.

There are very few terrestrial bacteria which can not withstand 50° C. for ten minutes, but the average marine bacterium is so thermosensitive that it might be inactivated by unduly prolonged exposure to the plating temperature of nutrient agar, 42° C. This psychrophilic thermosensitive characteristic of marine bacteria must be taken into consideration in dealing with them or in evaluating their importance in the world below sea level or their relation to man.

Importance of Bacteria in the Sea

Marine bacteria are not merely biological curiosities. In spite of their minute size, they can be characterized as the mighty mites of the deep in view of their far-reaching effects upon conditions in the sea. Although they are so small that individuals must be magnified nearly a thousand diameters before they can be seen, the standing crop of bacteria in the oceans of the world is estimated to be no less than ten million tons. If they multiply only once a day (and the generation time of some is known to be less than an hour), the annual crop of marine bacteria would exceed 3 trillion tons. These are minimum estimates

based upon an average bacterial population of ten bacteria per cc, each weighing an average of 5×10^{-13} gm.

However, it is not by their mass but by "their works that ye shall know them." And, indeed, we are much more interested in their activity than in the organisms as individuals. Experiments which have been designed to simulate conditions in the sea indicate that bacteria influence the composition and concentration of nitrogen, sulfur and phosphorus compounds as well as the organic content of sea water. Bacteria are present which can oxidize or reduce nitrogen and sulfur compounds; they liberate phosphate from phosphorus compounds, and they mineralize waste organic matter to give carbon dioxide and other plant nutrients.

It is due to their efficiency in mineralizing organic matter that the sea has been characterized as "the world's largest and most efficient septic tank." The organic content of the ocean is kept well below ten parts per million in spite of the extensive animal population and terrigenous pollution.

Bacteria are probably more important than any other group of organisms in reducing the oxygen tension of sea water. There are enough respiring bacteria present to utilize from 0.1 to 5.0 cc of oxygen per liter of water per year in different parts of the ocean. In certain localized regions where oxygen is not readily replaced from the atmosphere or by photosynthetic activity, they utilize the last trace of dissolved oxygen often causing extensive "oxygen deserts."

As a source of food bacteria are consumed by many animals both large and small. Not only do protozoa, copepods and other microscopic animals thrive on a bacterial diet; macroscopic animals, including mussels, oysters, tunicates and worms, can live indefinitely on bacteria.[8] Many animals dwelling at or near the bottom where bacteria are abundant probably derive much of their nutrition from the bacteria which they ingest.

[8] C. E. ZoBell, *Jour. Mar. Res.*, 1: 312–327, 1938.

Bacteria as Geological Agents

Through their effects on the chemical and physico-chemical conditions in bottom deposits bacteria exert a far-reaching influence on the diagenesis of sedimentary materials. As the principal dynamic agencies which alter the hydrogen-ion concentration they tend to precipitate or dissolve calcium carbonate (limestone), depending upon whether they increase or decrease the hydrogen-ion concentration. Bacteria are present in marine bottom deposits which can do either, the direction and magnitude of the change being largely a matter of organic, sulfate and nitrate content.

Under favorable conditions bacteria play a role in the deposition of bog iron and in the precipitation of manganese compounds. While there are specific bacteria which oxidize iron and manganese, the state of these metals in sedimentary materials is primarily a function of the oxidation-reduction potential. The latter is influenced by bacterial action.

Certain bacteria transform sulfur compounds, often giving rise to sulfides, free sulfur or sulfates. The decrease in the quantity and changes in the nature of organic matter in sedimentary materials in the initial stages of lithification are attributable primarily to the activities of the microflora.

Do Bacteria Produce Petroleum?

It is still indeterminate if bacterial activities play a role in the genesis of petroleum. In fact, the origin of petroleum is still a mystery, although it is the consensus of oil geologists that petroleum is derived from the transformation of organic matter at the bottom of the sea by either biological, chemical or geophysical agents; probably all three.

Bacteria possess certain unique properties by which they could contribute to the formation of petroleum and they are present in potential source beds of petroleum in significant numbers.

By reducing the oxygen, nitrogen and phosphorus content of organic matter, bacteria convert it into substances which are more petroleum-like. This is illustrated by the data summarized in Table 4. Very low oxidation-reduction potentials created by bacteria in certain environments are theoretically conducive to the formation and accumulation of hydrocarbons. Methane-producing bacteria occur in marine bottom deposits and it has been postulated that methane might be polymerized geophysically with the formation of higher hydrocarbons. It is not improbable that certain complex hydrocarbons are produced directly by bacterial action on marine sapropel.

TABLE 4
PROXIMATE ANALYSIS OF ORGANIC MATTER FROM DIFFERENT PETROLIFEROUS ENVIRONMENTS

Source of organic matter	Carbon	Hydrogen	Oxygen	Nitrogen	Phosphorus
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Marine sapropel ...	49	5	40	3.4	0.8
Recent sediments ..	58	7	34	2.2	0.6
Ancient sediments .	73	9	14	0.9	0.3
Petroleum	85	13	0.5	0.4	0.1

The occurrence of sulfate-reducing bacteria in oil-well brines and in petroliferous marine sediments, together with the accompanying lower sulfate-chloride ratio, suggests a relationship between these bacteria and the formation of petroleum. Bacteria which oxidize various waxes, oils and other hydrocarbons may be instrumental in the destruction of petroleum under certain conditions.

Disease-Producing Bacteria

Although marine bacteria are predominantly beneficial, there are a few which are pathogenic or disease-producing. Very few of the bacteria which inhabit the sea are pathogenic for marine animals, and as far as is known, none of them cause disease in man. Typhoid bacilli, the vibrio which causes Asiatic cholera and certain other human pathogens, may remain alive for a few days after being discharged into the sea, and hence sea water polluted by man is a potential source of danger, but fortunately such bacteria do not live long in the sea.

It is estimated that there are enough coliform bacteria discharged by the sewage effluents along the west coast of the United States each day to give over a hundred for every liter of water in the North Pacific Ocean if they were uniformly distributed. Yet one seldom finds coliform bacteria in the sea, except in bays or in the immediate proximity of sewage effluents, because they are destroyed so rapidly. However, in the latter places, untreated sewage may be very dangerous to bathers or consumers of shell-fish as well as esthetically offensive.

There is evidence that certain marine animals succumb to bacterial infections. However, the conditions of life in the sea are not conducive to the propagation of pathogens, although parasitic animals thrive there. As soon as a pathogen incapacitates his host, the latter almost immediately falls prey to the ever-present predator and the evidence is destroyed. There is no sanctuary in the sea for the ill or the old where only the fittest survive.

Any diseased fish or other animal which can no longer swim quite as fast as his companions is soon captured and probably eaten by a larger fish or other predator. Consequently the pathogen which incapacitates his host is destroyed also, unless the pathogen can attack the predator. Thus, to incapacitate his host, the pathogen is committing suicide.

Nevertheless, there are some marine pathogens which continue to take their toll of both plants and animals inhabiting coastal waters. A few years ago the lobster industry on the West Coast was threatened by a "soft shell" disease which killed large numbers of lobsters and made others unsuitable for human consumption.

In aquaria, experimental tanks and elsewhere in captivity, many marine animals have their resistance weakened to such an extent that they become victims of bacterial infections. Similarly, kelp, eel grass and other marine vegetation sometimes become infected with devastating pathogenic microorganisms when the resistance of the plants is lowered by storms, man's pollution or otherwise. Bacteria have been observed which injure the pneumatocysts or float bladders of kelp causing the kelp to sink to the sea floor, where it dies and rots. In general, though, while marine bacteria have a predilection for living associated with plants or animals as symbionts, commensals or epiphytes, they rarely injure oceanic hosts.

Sessile Habit of Bacteria

Contrary to popular conception, it is doubtful if many marine bacteria are planktonic or free-floating organisms. Most of them seem to occur attached to objects larger than themselves, such as plants or animals, settling particles of sediment, the remains of dead organisms or other particulate material. This peculiarity of marine bacteria has been attributed to the fact that organic matter is concentrated by adsorption upon solid surfaces where the bacteria can feed more efficiently when attached than when the bacteria are swimming or floating about in dilute nutrient sea water.

Some bacteria have a mechanism for attaching themselves to objects, some are stereotactic, others are adsorbed on solid surfaces and still others are able to grow only on bodies where nutrients are concentrated. Bacteria belonging to the latter category are especially abundant in sea water where organic nutrients are dilute.

Bacteria digest most of their food extracellularly or outside of their own bodies. They must render their food soluble by their digestive enzymes before the food can be ingested. Since there is less than 10 milligrams of organic matter per liter of sea water ordinarily, the bacteria have considerable difficulty in such a dilute medium trying to digest and ingest enough food to satisfy their nutrient requirements because it diffuses away from them about as fast as they can digest it. If, however, the bacteria are on a solid surface which will help to concentrate organic matter and to retard the diffusion of the soluble digested food away from them, they are able to thrive. Recent studies indicate that the solid surfaces also facilitate the orientation of the bacterial exoenzymes in the most advantageous position.

Fouling of Submerged Surfaces

This sessile sedentary habit of marine bacteria which causes them to localize on solid surfaces is sometimes inimical to man. Quite indiscriminately the bacteria grow on virtually all types of submerged surfaces, including ships' bottoms, hydroplane pontoons, water conduits and other man-made structures. After a few days' submergence in sea water the exposed surfaces are coated with a thin layer of bacteria along with other film-forming microorganisms.

The thin layer of film is rarely noticed unless one uses a high-powered microscope or other specialized analytical procedures, and by itself it is quite unobtrusive. However, the film attracts a myriad of tiny larval animals which permanently attach themselves to the surfaces. These attached animals, including barnacles, mussels, oysters, hydroids,

bryozoa and other "fouling" organisms, grow with surprising rapidity, and before many months they are clogging water conduits or seriously retarding the movement of vessels through the water.

There is evidence that bacteria influence the fouling of submerged surfaces in several ways: They discolor bright smooth surfaces which might otherwise discourage the attachment of fouling organisms. Bacteria serve as a source of food for the barnacle and his nefarious kin. They often protect the fouling organisms from the specific poisons in paints either mechanically, by adsorbing the poisons or by rendering the poisons inert by the production of hydrogen sulfide or other chemical substances. Under certain conditions they increase the alkalinity of the surface film, thereby favoring the deposition of the calcareous cement by which certain fouling organisms attach themselves. The bacteria produce plant nutrients which favor the growth of sedentary algae.

As much as 12 to 14 per cent. by weight of the fouling cumulation of ships' bottoms may consist of bacteria. We have isolated nearly fifty different kinds of sessile bacteria from such material, and other investigators are adding steadily to the list.

The so-called "fouling" organisms whose attachment is influenced by the primary film-forming microorganisms are most annoying and they necessitate the frequent drydocking of boats and the reconditioning or replacement of water conduits and other submerged marine structures at considerable cost. It is estimated that in times of peace, fouling organisms cost the United States Navy and merchant marine more than a hundred million dollars annually. In times of war the cost of fouling organisms is inestimable, considering how they retard the speed of vessels, increase fuel consumption and finally necessitate taking important vessels out of commission for cleaning in a drydock at a time when they may be urgently needed. The incrimination of bacteria and allied microorganisms as contributory agents dictates that they be taken into consideration in investigations designed to solve the problem.

Conclusions

Bacteria are widely distributed in ocean water and bottom deposits. Morphologically they resemble terrestrial bacteria, but they exhibit certain physiological characteristics and cultural requirements which are distinctive. Their sensitivity to temperatures exceeding 25° C., their ability to grow at low temperatures and their specific salt requirements are especially noteworthy.

Bacteria influence chemical, physicochemical, geological and biological conditions in the sea in many ways. Their possible relationship to the productivity of the sea, to the spoilage of marine products of commerce, to the genesis of petroleum and to the fouling of submerged surfaces are practical problems which command attention.

Further information on the bacterial population of the marine world will increase our understanding of oceanic phenomena and the marine environment besides contributing to our knowledge of bacteriology. Eventually it may become possible to apply such information to the control or amelioration of some of the harmful activities of bacteria and to take fuller advantage of those which are beneficial.

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PELAGIC LARVAL STAGES OF THE SAND CRABS EMERITA ANALOGA (STIMPSON), BLEPHARIPODA OCCIDENTALIS RANDALL, AND LEPIDOPA MYOPS STIMPSON

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Introduction

In a previous report (Johnson, 1940) the larvae of *Emerita analoga* were dealt with from the standpoint of dispersal of pelagic stages as correlated with and as indicators of water currents off the California coast. That report was made possible only through a detailed study of the pelagic developmental stages which provided a key to the exact identification essential to investigation of the distribution.

The present report will cover mainly the descriptive details of the larval developmental stages of which there are five.

A study of the early larval stages of two other less abundant sand crabs, namely *Lepidopa myops* Stimpson and *Blepharipoda occidentalis* Randall, was undertaken by the senior author, and is here reported briefly for comparison. This study was made in order to ascertain whether or not the larvae of these related forms are sufficiently similar to those of Emerita to cause confusion when piecing together the developmental series by collecting the later zoeal stages from the plankton as was done for Emerita. That no such confusion is possible is seen from the strikingly different structure of the zoeal stages of each as shown in the figures.

Emerita analoga

Emerita analoga is abundant in the intertidal zone of many sandy beaches along the coast of southern California and according to Schmitt (1935) its range of distribution is from Oregon (Holms); Drake Bay, California, to Magdalina Bay, Lower California; and from Salvery, Peru, to Lota, Chile. A second Pacific species, *Emerita rathbunae* Schmitt, occurs from La Paz, Lower California, to Capon, Peru, but because of the remoteness from our region its pelagic larvae are not likely to be confused with larvae of *Emerita analoga* caught in our waters.

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The larvae of two species of Emerita, namely *Emerita talpoida* of the eastern North American coast and *Emerita asiatica* of the Asiatic coast, have previously been investigated, both under the generic name *Hippa*. Of these, the former was studied incompletely by Smith (1877) and in greater detail by Faxon (1879) and the latter in detail by Menon (1933), who also makes comparisons between the two species.

As might be expected within the same genus, the structure of these larvae is very similar to that of our local species. The major differences will be noted in the course of discussion.

The eggs are carried attached to the abdominal appendages during the incubation period and the larvae, which quickly become pelagic, hatch in the first zoeal stage. A figure of this stage and also early cleavage stages of the eggs has been given by Johnson and Snook (1927).

Distribution of Larvae

In Table I is given an analysis of the occurrence of Emerita larvae for cruises covering the period April 8 to December 18. The seasonal distribution here suggested would indicate that the bulk of larvae are

TABLE I

Frequency of occurrence of Emerita larvae taken during the routine cruises of the "E. W. Scripps" for periods indicated

1938	Number of stations	Per cent successful for larvae	Zoea I	Zoea II	Zoea III	Zoea IV	Zoea V
April 8–12	7	0.0					
June 7–18	25	4.0			1		
August 16–26	34	38.0	9	30	12	12	1
October 26–November 5	28	36.0			6	25	
December 9–18	27	22.0			4	17	1

hatched during July and early August. This appears also to be in keeping with the conclusion of MacGinitie (1938) that the height of the mating season falls in May and June, though the duration of egg carriage by the female has not been determined. The wide distribution (Figure 1) indicates that the pelagic period is a relatively long one enabling the larvae to become dispersed and transported from their place of origin in the intertidal zone to distances of over a hundred miles seaward.

It is interesting that somewhat greater numbers of larvae were taken at stations relatively far from the coast, and the frequency of separate stations yielding one or more larvae is about the same here as in the

inshore area. These offshore stations fall in an extended area paralleling the coast as indicated by the line in Figure 1 enclosing locations where a total of six or more larvae have been taken. This is particularly striking since it is contrary to expectations in view of the coastal origin of the larvae and the diminution of numbers expected to accompany offshore dispersal. In seeking an explanation, it should be pointed out that it has been shown (Sverdrup and Fleming, 1941) that during

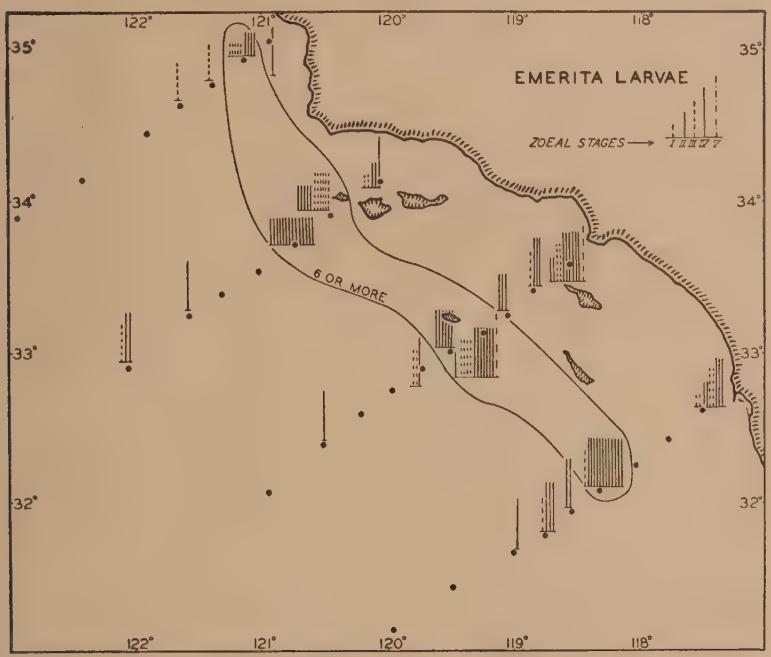

FIGURE 1

this period there exists a body of upwelled and mixed water streaking southward off-shore corresponding roughly to the area abundant in Emerita larvae. These waters are also notably productive in phytoplankton (Sverdrup and Allen, 1939) and in zoöplankton (Johnson, unpublished). Certain marine birds, e.g., the black-footed albatross, occur in greatest numbers in a long narrow band corresponding roughly to this area of increased organic production (Miller, 1940). It may therefore be suggested that, perhaps, the abundant plankton food supply makes this also a better region for survival of larvae than is true of the waters between this region and the immediate coast.

Studies of the ecology and offshore distribution of Emerita larvae in other areas are not available for comparison.

Methods

In the preliminary study, oviferous females were collected on the beach and kept in aquaria until the eggs were about to hatch, which is indicated by a change of color in the eggs from a bright coral red to a dull grayish-brown. At this stage an entire appendage with its egg mass was removed and placed in a glass culture chamber constructed from a 60 by 19 mm. shell vial. The bottoms of the vials were removed and replaced by a piece of number 8 × (average mesh aperture of 0.21 mm.) bolting cloth held in place by a rubber band. The tops were closed in a similar manner. Several culture chambers stocked with eggs were then placed end on end in a vertical glass tube approximately 45 centimeters long and 22 millimeters in diameter, or just sufficient to allow the shell vials to slip into place. The tube was held in a vertical position by a clamp and ringstand. Aerated sea water, pumped from the sea, was allowed to pass upward through the tube and culture chambers and overflow from the top. The rate of flow was regulated by screw clamps such that the eggs were almost held in suspension by its force. In this manner hatching usually occurred within a period of 24 hours and approximately half of the larvae hatched by this method were already free-swimming by this time. The young zoea are very helpless until their maxillipeds and their posterior setae are fully extended, for it is by means of these that the larvae are enabled to swim. Zoea which were hatched in the above manner lived without attention from six to eight days in the same culture chamber.

Feeding the larvae diatoms, ground Ulva or fresh plankton in the tubes failed to lengthen the period of life. Thus it was not possible to rear the larvae in the tubes sufficiently long to enable them to pass into the second zoeal stage; nevertheless, the method was the most satisfactory in caring for the eggs and bringing about a high percentage of hatching.

The best success in rearing the larvae beyond this age was realized in cultures consisting of a small number of individuals. Two free-swimming zoea were kept alive in a Syracuse watch glass for 34 days. The water was changed daily by means of a pipette and a portion of a fresh plankton haul, which had been strained through number 8 × bolting cloth to remove large and possibly harmful organisms, was fed daily. Similar success was also obtained with two zoea placed in a finger bowl and kept at approximately 18° C. in a constant temperature room which was totally dark. The water was kept in motion by a mechanical rocking

table, which was in constant operation throughout the period. One of the two larvae died on the twenty-fourth day without further change. The remaining one lived an additional ten days in which time it entered the second zoeal stage. Two long spines had developed from the posterior lateral margins of the carapace. These extended obliquely downward at an angle of about 45 degrees from the dorsoventral line and were approximately as long as the carapace was wide. The rostral spine had become greatly elongated. This specimen, unfortunately, soon died, but it showed the distinguishing features of the second zoeal stage and provided a key to following through the subsequent stages from specimens collected from the plankton as indicated below.

Some zoea of stages I and II were obtained from routine plankton hauls made with small nets at the Institution's pier and also from occasional samples collected at stations five to ten miles off Point Loma. Stages IV and V were, however, absent from all of these near-shore collections. The later stages and also most of stages II and III were collected off-shore, mainly in vertical plankton hauls taken from 200–0 meters with a 70 cm. net aboard the research vessel, "E. W. Scripps," at four lines of stations extending seaward to distances of 150 to 180 miles off the coast from San Luis Obispo to San Diego (Fig. 1).

Description of Zoeal Stages

Stage I (Plate I, Figure 1)

Average sizes:

Maximum width of carapace	.53 mm.
Maximum length of carapace	.70 mm.
Length of abdomen including telson	.72 mm.
Length of rostral spine	.20 mm.

The average sizes given for this and all subsequent stages except Stage V are based on measurements of ten or more specimens. In Stage V only five specimens were available. The carapace measurements are used in preference to total length because the inclusion of the abdomen in the latter is not ordinarily practicable owing to its being strongly flexed in a position making exact measurement difficult.

Stage I is essentially similar to the first stage in the development of *E. talpoida* as given by Faxon and also that of *E. asiatica* as described by Menon. It is characterized by a short rostral spine and by the absence of lateral spines on the carapace. Slight thickenings mark the region where the latter will appear in the second zoeal stage. The eyes are conspicuously stalked and project laterally at right angles, slightly beyond the margin of the carapace. The carapace in this and subsequent stages is translucent, colorless and of uniformly smooth texture.

First Antennae (Plate II, Figure 1).—These are thick, short, unjointed processes bearing three aesthetes at the distal ends. No rudiments of the secondary flagellum were observed until the post-zoeal stage as was found also by Smith and Menon in *E. talpoida* and *E. asiatica* respectively.

Second Antennae (Plate II, Figure 6).—The antennae are each terminated by an outer spine-like process with a thickened base that is continuous with the main body of the antenna. There is also a smaller dentiform process and from near its base there issues a small setose spine which remains essentially unchanged throughout the following zoeal stages.

Mandibles (Plate II, Figure 11).—The mandibular blades bear from six to ten sharp teeth arranged in a row, the ventral tooth being always much heavier and longer than the others. These appendages remain practically unchanged except for general growth throughout the zoeal stages.

During metamorphosis to the adult state, the mandibular blades are lost. This is doubtless correlated with the radical change that occurs in feeding habit at this time, for in the adult state the animal has secondarily adopted the habit of remaining quiescent in the sand while the plumose antennae screen microscopic food from the outgoing waves.

First Maxillae (Plate II, Figure 13).—These are similar to the first maxilla in *E. asiatica,* but have one less seta on the endopod than in *E. talpoida.* The exopod is terminally cleft and each branch bears a stout setose spine. On the newly hatched zoea these spines are deeply imbedded. Branching off about half way down the outer margin of the exopod is a small lobe armed with a single long seta. The endopod bears a group of three partially imbedded setae. A single small seta is found proximal to this group and remains unchanged throughout the zoeal development. This was also found to be true of *E. asiatica* by Menon.

Second Maxillae (Plate II, Figure 16).—The second maxillae are similar in shape and arrangement to *E. asiatica* and *E. talpoida.* However, the number of setae on the scaphognathite differs from both of these species. Faxon shows a different arrangement of setae on the

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PLATE I

Emerita analoga—zoeal stages I–V

FIGURE 1. Stage I (enlarged scale).
FIGURE 2. Stage I (same scale as following stages).
FIGURE 3. Stage II.
FIGURE 4. Stage III.
FIGURE 5. Stage IV.
FIGURE 6. Stage V.

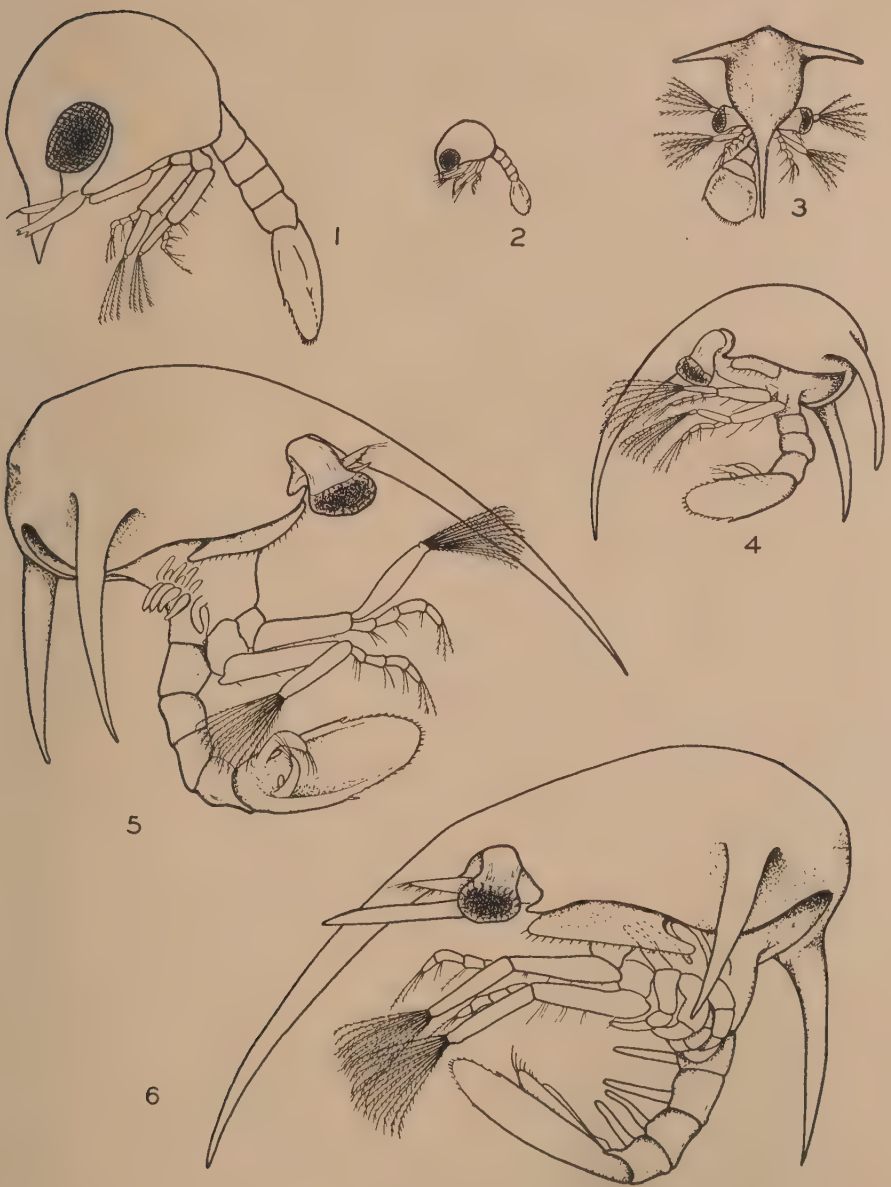

PLATE I

protopod for *E. talpoida*. The appendage of *E. analoga* is divided into two parts; the protopod bears a cluster of three short rudimentary setae accompanied by a fourth seta somewhat removed down the median margin. This isolated seta remains unchanged throughout the remainder of the developmental stages as is also the case in *E. asiatica*. The scaphognathite bears nine to ten setae along its anterior-outer margin. The posterior and inner margins are naked, differing in this respect from *E. talpoida*.

First Maxillipeds (Plate II, Figure 18).—These are essentially similar to those figured for *E. asiatica*. They are composed of a very short coxopod with a long basipod about equal in length to that of the endopod, including the three distal setae. The basipod bears six setae along the inner margin. The endopod consists of four segments each bearing setae. The first segment has three setae just below the joint, each of the next two has two in the same position. The distal segment has four setae of unequal length, the outermost two being the longest. No spinules as mentioned by Menon for *E. asiatica* were observed. The exopod consists of an elongated segment slightly longer than the endopod and a very short terminal segment bearing four long plumose setae.

Second Maxillipeds.—These are very similar to the first maxillipeds except that the distal segment of the endopod is relatively longer. The basipod is more slender and has less setae on its inner margin. The segmentation in both of these appendages remains the same throughout the later stages, the only point of difference, other than general growth, being in the number of setae on the distal segment of the exopod as

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PLATE II

Emerita analoga—zoeal appendages

FIGURES 1 to 5. First antenna, Stages I to V.
FIGURES 6 to 10. Second antenna, Stages I to V.
FIGURE 11. Mandible, Stage I.
FIGURE 12. Mandible, Stage V (reduced scale).
FIGURE 13. First maxilla, Stage I.
FIGURE 14. First maxilla, Stage III.
FIGURE 15. First maxilla, Stage IV.
FIGURE 16. Second maxilla, Stage I.
FIGURE 17. Second maxilla, Stage V.
FIGURE 18. Maxilliped, Stage I.
FIGURE 19. Maxilliped, Stage V.
FIGURE 20. Telson, Stage I.
FIGURE 21. Uropod, Stage V.
FIGURE 22. Uropod, Stage IV.
FIGURE 23. Uropod, Stage III.
FIGURE 24. Arrangement of mouth parts, Stage V.

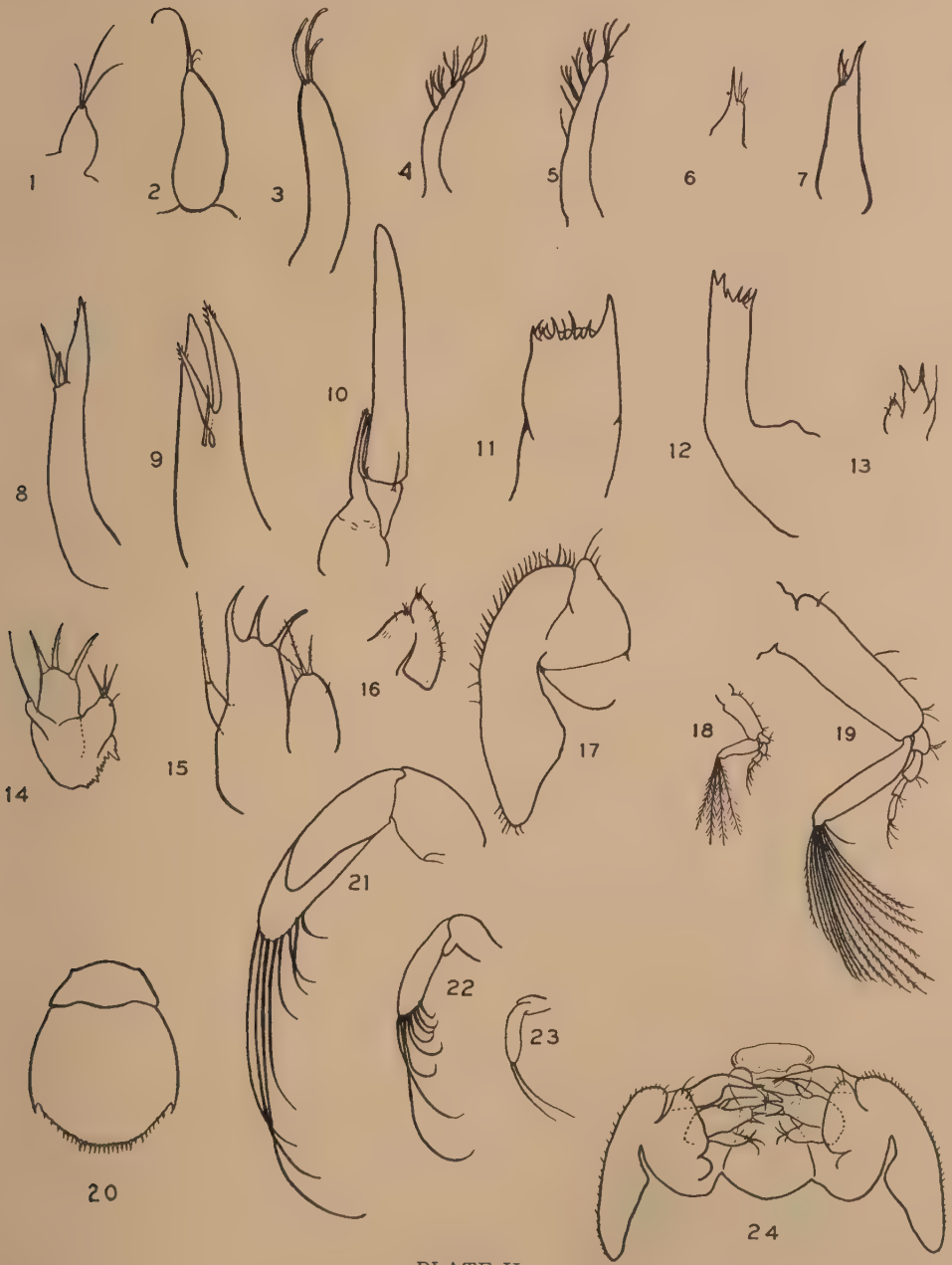

PLATE II

mentioned later. No rudiments of other thoracic appendages were found posterior to the second maxillipeds.

Abdomen.—The abdomen is composed of four free segments and the telson; the fifth and sixth segments are consolidated in the telson according to Smith. Proceeding from anterior to posterior the individual segments become shorter and markedly broader. No appendage rudiments appear either on the telson or on the abdominal segments until a later stage.

The telson (Plate II, Figure 20) agrees with *E. talpoida* but differs from the Asiatic species in that its width is slightly in excess of its length. However, there is a good deal of individual variation in this respect and in some it is fully as long as wide. The number of spines on the posterior margin is the same as that given for both *E. asiatica* and *E. talpoida.* This number, twenty-six, remains constant throughout the later stages.

STAGE II (Plate I, Figure 3)

Average sizes:

Maximum width of carapace	0.8 mm.
Maximum length of carapace	1.0 mm.
Length of rostral spine	1.2 mm.
Length of lateral spine	1.0 mm.

Stage II is characterized by the presence of two long, stout, posteriolateral spines nearly as long as the carapace is wide. These spines together with the rostral spine, which has increased to over three times the length attained in the preceding stage, form a tripod upon which the zoea is supported when resting on its ventral side. No additional appendages make their appearance in this stage. The carapace is no longer circular when viewed dorsally, but is now considerably longer than wide, and the eye stalks are projected more forward than in the previous stage.

First Antennae (Plate II, Figure 2).—Instead of three setae of about the same size, as found in Stage I, there are now two very much reduced slender setae and one long stout aesthete. *E. analoga* differs in this respect from both *E. asiatica* and *E. talpoida.*

Second Antennae (Plate II, Figure 7).—As in Stage I.

Mandibles.—As in Stage I.

First Maxillae.—The first maxillae now have six plumose setae issuing from the tips of the exopods.

Second Maxillae.—One additional seta is present on the margin of the scaphognathite.

First and Second Maxillipeds.—Each of these appendages now has six plumose setae on the tips of the exopods. No rudimentary appendages were present posterior to the maxillipeds.

Telson.—The telson is longer than wide. The number of spines on the proximal margin remains as in Stage I, but the number of denticles between the spines has increased.

STAGE III (Plate I, Figure 4)

Average sizes:

Maximum width of carapace	1.3 mm.
Maximum length of carapace	1.6 mm.
Length of rostral spine	2.1 mm.
Length of lateral spine	1.4 mm.

Stage III differs from the preceding stage mainly in size and in the presence of uropods on the telson. Two more setae make their appearance on the distal ends of the exopods of the first and second maxillipeds. The eyestalks have enlarged and now extend at an oblique angle, downward and forward. This is a continuation of the shift from a lateral position in the early stages to a position parallel to the median line as in the adult. In agreement with Menon, it appears that Smith's description and figures of what he (Smith) calls Stage two in the development of *E. talpoida,* correspond more closely to Stage III.

First Antennae (Plate II, Figure 3).—The first antennae have grown slightly longer and more slender, and bear three terminal aesthetes of unequal length.

Second Antennae (Plate II, Figure 8).—These are now slightly longer than the first antennae.

Mandibles.—As in preceding stage.

First Maxillae (Plate II, Figure 14).—As in preceding stage.

Second maxillae have eleven setae on the outer margin of the scaphognathite, being an increase of one over the previous stage.

First and second maxillipeds now bear eight plumose setae on the tips of the exopods, but there is no other change except general growth.

No other thoracic appendages appear posterior to the maxillipeds. Both Smith and Menon found rudiments of such appendages in *E. talpoida* and *E. asiatica* respectively. These rudiments may occur just prior to the molt to the fourth stage.

The *abdomen* is still without pleopods.

The *telson* is unchanged except for development of uniramous rudiments of uropods on its anterior ventral side (Plate II, Figure 23). These are each composed of a short unjointed basal segment and a long, curved, flattened lobe extending from it. This lobe becomes the exopod of the appendage in the later stages and is tipped with two long and one short setae.

STAGE IV (Plate I, Figure 5)

Average sizes:

Maximum width of carapace	2.0 mm.
Maximum length of carapace	2.4 mm.
Length of rostral spine	3.9 mm.
Length of lateral spine	2.2 mm.

This stage shows the first evidence of additional thoracic appendages and rudimentary pleopods on the abdomen. The number of plumose setae on the exopods of the first and second maxillipeds has increased to 16.

First Antennae (Plate II, Figure 4).—In addition to the three terminal aesthetes found in stage three, there are now eight more, distributed in two groups of three each and one group of two located on the inner side. Smith found only five or six in *E. talpoida* while Menon shows five for *E. asiatica.*

Second Antennae (Plate II, Figure 9).—These have grown considerably and the flagellum is now nearly as long as the dentiform processes. A small setose spine is situated at the base of the inner dentiform process. This appendage agrees generally with its equivalent in *E. talpoida* and *E. asiatica,* the difference being in the growth of the flagellum and in the number of spines on the dentiform processes.

Mandibles as in preceding stage.

First Maxillae (Plate II, Figure 15).—The inner lobe has elongated somewhat and bears one more small spine slightly proximal to the group of three on the distal end.

Second maxillae have now each 29 setae on the scaphognathite; an increase of 18 over the number in Stage III.

First and second maxillipeds differ from Stage III only in that the number of long plumose setae on the tips of the exopods have increased to 16. *E. asiatica* and *E. talpoida* each possess a total of only ten setae on these parts, which corresponds to lower Stage IV (see below) in *E. analoga.*

The additional thoracic appendages that are more fully developed in later stages appear as rudiments just posterior to the second maxillipeds. These consist of a third pair of maxillipeds and four pairs of legs which develop into walking legs in the first post-zoeal stage. Rudimentary gills appear above each pair of legs and are partially hidden by the margin of the carapace.

The *abdomen* is unchanged except for the presence of slight projections on the first four segments which were taken to be rudiments of the pleopods.

Uropods (Plate II, Figure 22).—The uropods have developed considerably over the preceding stage. The setae on the ends of each exopod have increased to eight which is three more than found in *E. asiatica* and four more than were reported in *E. talpoida.* These setae are of unequal length as was also reported for the other two species. Rudimentary endopods have appeared.

The *telson,* now narrower and longer, has no increase in number of marginal spines but the number of denticles between these has increased slightly. Three to six denticles were found between the spines near the median line and 25 between the last spine on the ventral margin and the large spine at the angle.

It should be mentioned here that a number of specimens were examined that appear to be intermediate between Stage III and Stage IV, being somewhat nearer the latter and may therefore, for convenience, be called "Lower Stage IV." It is not clear whether they represent a distinct instar between the above stages or are simply variables in Stage IV. In size they are comparable to the smaller specimens of that stage. The distinguishing features of Lower Stage IV are: ten setae on the tips of the exopods of the maxillipeds, a small precursory rudiment of the second antennal flagellum of later stages, absence of rudimentary thoracic appendages back of the second maxillipeds and no rudimentary endopod buds on the uropods.

STAGE V (Plate I, Figure 6)

Average sizes:

Maximum width of carapace	2.6 mm.
Maximum length of carapace	3.5 mm.
Length of rostral spine	4.2 mm.
Length of lateral spine	2.1 mm.
Length of abdomen, including telson	3.6 mm.
Width of telson	1.9 mm.
Length of telson	2.8 mm.

Stage V, which is the last zoeal stage, shows its greatest advancement over preceding stages mainly in the development of pleopods on all four segments of the abdomen and in the increased development of the thoracic legs. The flagellum of the second antenna is now conspicuous from a dorsal view of the animal.

First Antennae (Plate II, Figure 5).—The aesthetes have increased to fifteen, quite clearly arranged in groups: a terminal group of three long and one short, followed proximally by three successive groups consisting of three long aesthetes in each group. These in turn are followed by two solitary aesthetes. This arrangement is similar to that

found in the descriptions of Smith and of Menon for their species, but the numbers are greater by five in *E. analoga*.

Second Antennae (Plate II, Figure 10).—The flagellum has elongated to about three times the length of the dentiform processes. The distinct segmentation of the flagellum and of the first antenna as shown for *E. asiatica* was not in evidence but this condition becomes obvious only shortly before the zoea moults to the first post-zoeal stage.

Mandibles (Plate II, Figure 12).—No change.

First maxillae as in preceding stage.

Second maxillae (Plate II, Figure 17).—These now have 33 setae along the outer margin of the scaphognathite which agrees with the asiatic species. There are no setae on the inner margin.

First and second maxillipeds (Plate II, Figure 19).—These appendages show no increase in the numbers of setae on the exopods, the number remaining 16. The asiatic species has an increase from 10 in Stage IV to 12 in Stage V.

The other thoracic appendages are imperfectly segmented; the third pair of maxillipeds having three segments, and the legs each having a three-segmented basis and a one-segmented exopod, and a very rudimentary endopod.

The pleopods show no evidence of segmentation or other differentiations. They are still uniramous.

Uropods (Plate II, Figure 21).—The endopod is now approximately three-fourths the length of the exopod. The latter bears eight curved setae of unequal lengths. The endopod is unarmed. These appendages agree with the descriptions *E. talpoida* and *E. asiatica* except for the number of setae, the former having six present in this stage and the latter seven.

No attempt was made to rear the first post-zoeal stage from the fifth zoeal larva, and the numerous small specimens collected on the beaches were not studied sufficiently to warrant a detailed report. In view of the few fifth zoeal stages found in the plankton, and none of these being

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PLATE III

Blepharipoda occidentalis—first zoea

FIGURE 1. Mandible.
FIGURE 2. First maxilla.
FIGURE 3. Second maxilla.
FIGURE 4. Larva, lateral.
FIGURE 5. Larva, dorsal.
FIGURE 6. Second antenna.
FIGURE 7. First antenna.
FIGURE 8. Second maxilliped.

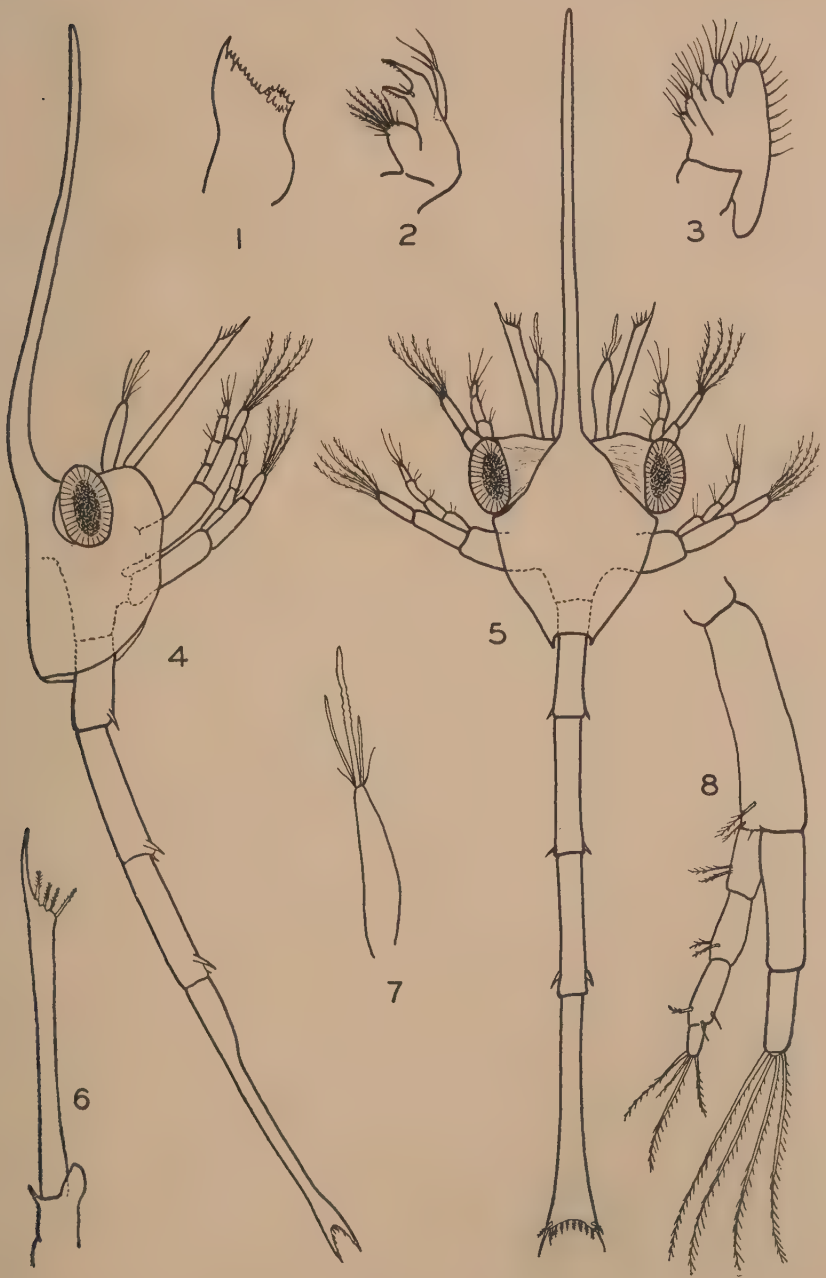

PLATE III

taken in the immediate vicinity of the beaches, it is a most profound mystery how these scattered planktonic stages or post-zoeal stages become sufficiently aggregated to account for the thousands of early post-zoeal stages constituting the large swarms found buried in the intertidal sands.

Blepharipoda occidentalis and Lepidopa myops

The adults of these two species occur in small numbers on sandy bottoms along the coast of southern and lower California. Though this study of their pelagic larval stages is incomplete, it is sufficient to enable distinguishing them from those of Emerita, and to make possible their identification for future studies on larval dispersal through water movements off the west coast.

The first zoeal larvae of Blepharipoda and Lepidopa were readily obtained by confining gravid females in small aquaria with sand and supplied with running sea water until the larvae hatched from the eggs which they carried. These stages are found sparingly also in the inshore plankton. Additional verification of the identity of the first stage of the former was also made from larvae reared by Mrs. S. Davis. No later larval stages have been obtained through rearing, but a plankton sample taken from 200 to 0 meters 30 miles off San Diego yielded a late zoeal stage of Blepharipoda.

Contrasted with the first zoea of Emerita, the corresponding stages of these sand crabs are strikingly distinctive. In Blepharipoda this is clearly reflected in the late stage as shown below, and the same is doubtless true also of Lepidopa. In Emerita it was necessary to know the anatomy of the second zoeal stage before the distinguishing features common to the later stages were recognizable. In that species the structure of the telson is the only striking characteristic carried forward to the successively older stages.

Blepharipoda occidentalis—first zoea (Plate III, Figures 1–8)

Carapace length not including spines	1.3 mm.
Carapace width	1.0 mm.
Rostrum	2.5 mm.
Abdomen including telson	3.5 mm.

Plate IV

Blepharipoda occidentalis—late zoea

Figure 1. Larva, dorsal (appendages omitted).
Figure 2. First antenna.
Figure 3. Second antenna.
Figure 4. Larva, lateral.
Figure 5. Urosome with uropod.
Figure 6. Third maxilliped.

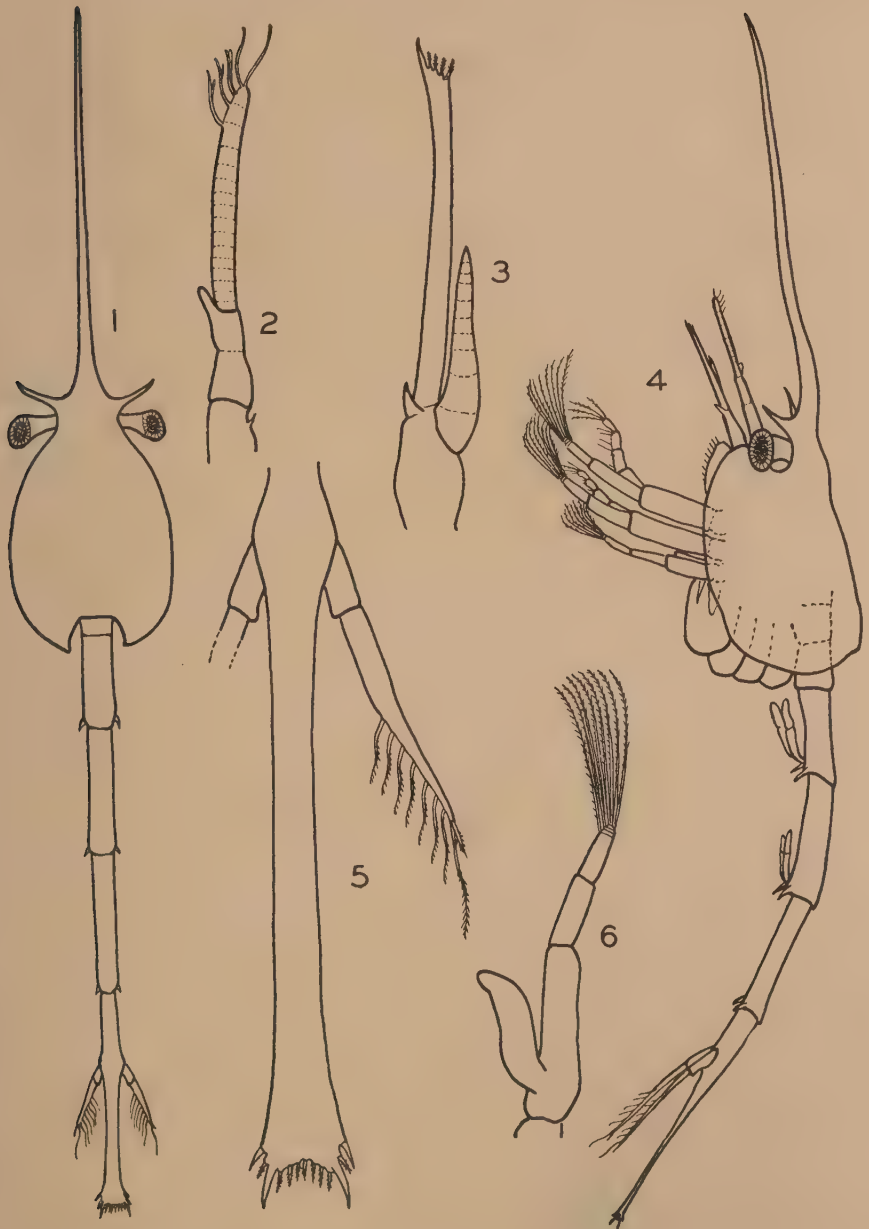

PLATE IV

The general features which sharply distinguish the larvae of this species from the corresponding stage in Emerita are the long rostral spine, the long, slender abdomen, and the narrow telson. It is separated from Lepidopa by the absence of lateral spines and by the character of the telson. Other details of the appendages as given below are also distinctive.

First Antennae (Plate III, Figure 7).—These appendages each bear a group of three terminal aesthetes and two small setae. They are relatively indistinctive in the three species discussed.

Second Antennae (Plate III, Figure 6).—These are very characteristic, each with a long distal segment, i.e. the scale or exopod, terminating in a curved spine-like process and four shorter setose spines on the inner side. The flagellum or endopod appears as a short rounded knob.

The *mandibles, first maxillae* and *second maxillae* (Plate III, Figures 1, 2 and 3) are in general not distinctive from the other species, though all are less rudimentary than in Emerita.

First and *second maxillipeds* are similar (Plate III, Figure 8). The exopod consists of two long segments and a very short terminal segment. Thus the segmentation differs from the other two species in that Emerita has but one distinguishable segment while Lepidopa has one long and two short segments. In each species however there are four long terminal setae. The endopod consists of four segments as in Emerita.

The *telson* (Plate III, Figure 5) is very narrow, with a thin concave posterior margin armed with two lateral smooth spines between which is a row of eight shorter setose spines. On the dorsal surface of the telson a short setose spine is situated at the base of each of the smooth spines.

Late zoea (Plate IV, Figures 1–6)

Carapace length not including rostrum	4.5 mm.
Carapace width	3.0 mm.
Rostrum	6.5 mm.
Abdomen including telson	10.0 mm.

PLATE V

Lepidopa myops—first zoea

FIGURE 1. Mandible.
FIGURE 2. First maxilla.
FIGURE 3. Second maxilla.
FIGURE 4. Larva, lateral.
FIGURE 5. Larva, dorsal.
FIGURE 6. First antenna.
FIGURE 7. Second antenna.
FIGURE 8. Second maxilliped.

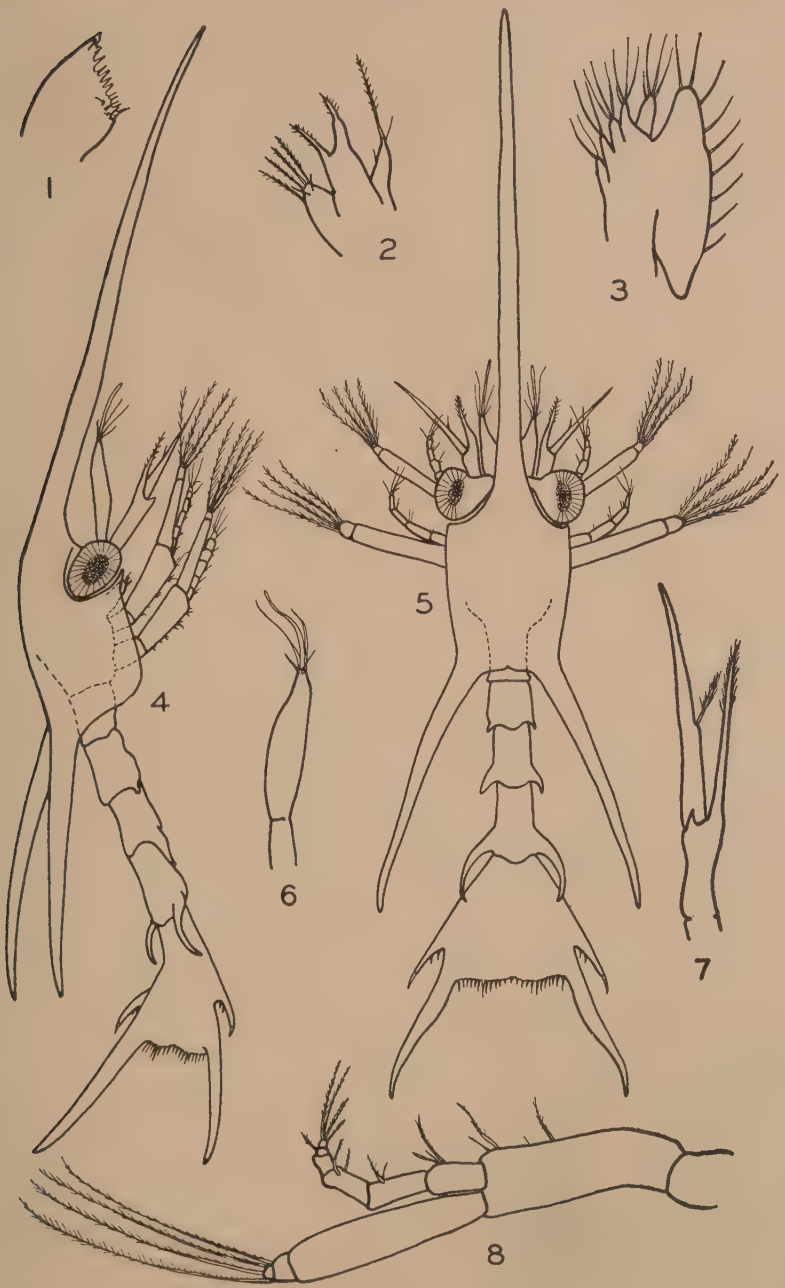

PLATE V

The most pronounced differences between this and the first zoeal stage are found in the development of (1), a pair of strong anteriorly directed spines on the carapace at the base of the rostrum; (2), a pair of slender uropods on the telson; and (3), third maxillipeds and rudiments of other thoracic and abdominal appendages as shown in Plate IV, Figure 4. The number of terminal setae on the exopod of the first, second, and third maxillipeds is eight. The scale or exopod of the second antenna has not changed but the flagellum has increased in length and shows segmentation under the skin. The telson armature has changed only by addition of a spine at each side of the posterior lateral angles. The eyes have become relatively much smaller and have very slender stalks, presaging the adult condition which perhaps will follow the next moult.

Lepidopa myops—first zoea (Plate V, Figures 1–8)

Carapace length not including rostral or lateral spines	1.2 mm.
Carapace width	0.8 mm.
Rostrum	2.8 mm.
Abdomen including telson	2.2 mm.

Distinguishing features characterizing this stage are: (1), the long rostrum; (2), carapace with long posterior lateral spines directed backwards and reaching well beyond the last abdominal segment; (3), relatively broad abdomen with conspicuous lateral spines which on the last abdominal segment are especially strongly developed; and (4), the very broad triangular telson provided with two pairs of heavy lateral projections between the larger of which is a series of 24 small spines. Smaller but distinctive features are found in the second antenna (Plate V, Figure 7) and the first and second maxillipeds (Plate V, Figure 8) the endopod of which consists of six distinct segments.

LITERATURE CITED

Faxon, Walter, 1879. On some young stages in the development of Hippa, Porcellana and Pinnixa. *Bull. Mus. Comp. Zool. Harv.*, **5**: 253–268.

Johnson, Martin W., 1940. The correlation of water movements and dispersal of pelagic larval stages of certain littoral animals, especially the sand crab Emerita. Sears Foundation: *Jour. Marine Res.*, **2**: 236–245.

Johnson, M. E., and H. J. Snook, 1927. Sea shore animals of the Pacific coast. Macmillan & Co., pp. 659.

MacGinitie, G. E., 1938. Movements and mating habits of the sand crab, Emerita analoga. *Amer. Mid. Nat.*, **19**: 471–481.

Menon, M. Krishna, 1933. The life histories of decapod crustacea from Madras. *Bull. of the Madras Gov. Mus., New Ser.*, **3**: No. 3; 1–45.

Miller, Loye, 1940. Observation on the black-footed albatross. *The Condor*, **42**: No. 5; 229–238.

SCHMITT, WALDO L., 1935. Crustacea Macrura and Anomura of Porto Rico and the Virgin Islands. *Sci. Sur. Porto Rico and Virgin Is., New York Acad. Sci.*, **15**: part *2*; 125–227.

SMITH, SIDNEY I., 1877. Early stages of Hippa talpoida, with a note on the structure of the mandibles and maxillae in Hippa and Remipes. *Trans. Conn. Acad. Sci.*, **3**: 311–343.

SVERDRUP, H. U., AND W. E. ALLEN, 1939. Distribution of diatoms in relation to the character of water masses and currents off Southern California in 1938. Sears Foundation: *Jour. Marine Res.*, **2**: 131–144.

SVERDRUP, H. U., AND R. F. FLEMING, 1941. The waters off the coast of Southern California, March to July 1937. *Bull. Scripps Inst. Oceanog.*, Univ. of Calif., **4**: 261–378.

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THE ERRORS OF THE SEDGWICK-RAFTER COUNTING CHAMBER IN THE ENUMERATION OF PHYTOPLANKTON*

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The study of the distributions and fluctuations in numbers of marine phytoplankton is an important field of oceanography and of biology in general. These microscopic, free-floating plants not only serve as an important food source of all life in the sea but are also extremely useful indicators of the physical and chemical conditions of their environment. At the present time one common method of studying these distributions is to estimate the average number of organisms per liter of the water mass under consideration. This estimation of population size is obtained from the microscopic enumeration of the organisms in one or more samples and, therefore, will be in error by an amount which depends upon the particular method used, the size and number of the samples, and the uniformity of distribution of the organisms in the water.

These errors of estimation are of interest to the phytoplanktologist for two reasons. In the first place, their determination should help the enumerator in deciding how large a fraction of the catch must be counted. This is of considerable practical importance as the actual enumeration is an exceedingly time consuming process. In the second place, the determination of the magnitudes and causes of these errors should facilitate their elimination or reduction. It is true that at the present time we are interested mainly in the relative orders of magnitudes of phytoplankton populations and that a relatively large error will not appreciably lessen the value of these counts. It is, however, equally true that a large error obscures many of the less obvious correlations and as our knowledge of the sea and its inhabitants increases, we shall be directing our attention more and more to these less obvious relationships. Every reduction in the error of quanti-

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tative plankton methods will, therefore, increase the potential usefulness of these methods and of the data obtained by them.

At the Scripps Institution of Oceanography the enumeration of phytoplankton is accomplished by counting the organisms in a quotal part of the water sample by means of the Sedgwick-Rafter counting chamber. The scope of this paper is not, however, limited to the Sedgwick-Rafter chamber. The basic questions considered are identical in all methods of enumerating phytoplankton.

It is, of course, impossible to determine, by sampling methods, the exact size of the phytoplankton populations in the sea. The best that one can hope to do is to be able to state, with a high degree of probability, the range within which the true value lies or, what is the same thing, the maximum per cent error. Inasmuch as the error of any count varies with the size and composition of the sample, this cannot be accomplished by simply determining the average variation between repeated counts of several samples and applying the resulting figure to all future counts. Furthermore, even if the average errors of different samples were the same, there would be little assurance that the error of any one sample would be equal to or less than the average error. The only solution lies in the application of probability or statistical theory. Certain statistical characteristics of the phytoplankton populations must be determined in order to predict the maximum variation that can be expected. In phytoplankton work it is impractical to calculate these characteristics for each sample and, therefore, characteristics will have to be used for this material that are common to all the populations encountered so that they can be either determined once and for all or can easily be approximated for each sample.

The theoretical treatment of the errors of phytoplankton counts is greatly complicated by the fact that colonial forms commonly predominate in phytoplankton populations. The frequency distribution for total cells is, therefore, the product of two other frequency distributions: the distribution of occurrences and the distribution of cells per occurrence. By total cells is meant the total number of cells regardless of whether or not they occur united in colonies while the term occurrences designates the number of colonies and individual non-colonial cells. *A priori*, the frequency distribution of occurrences can be expected to be very similar to the Poisson distribution. This is readily seen by considering the Sedgwick-Rafter slide to be divided up into a very large number of small areas each delimited by the field of the microscope. The probability, then, of any one particular organism being in one certain field is extremely small and the main condition for the Poisson distribution is fulfilled. The outstanding characteristic of the Poisson distribution is that the mean is equal to the variance. This brings about a great simplification in theoretical treatment and offers a practical solution for calculation difficulties. For this reason the errors of occurrences are dealt with first.

Occurrences can be expected to follow the Poisson distribution only if the sole source of variation is that due to random distribution. Any additional source of error will bring about an increase in the total variance and a corresponding deviation from Poisson distribution. In Sedgwick-Rafter counts of phytoplankton, there are two possible sources of additional error; namely, the clumping of colonies (cf., Littleford, Newcombe and Shepherd, 1940) and errors in filling the chamber. The effects of these sources of variation must be separated from one another and their relative effects upon the total variation determined in order to tell whether or not it is worthwhile to take steps to reduce or eliminate them. The filling error will be operative only in comparisons of counts made on different mounts and so can be determined by comparing an estimate of the total variance obtained from counts of the same mount with an estimate obtained from counts of different mounts. The variation due to clumping can be determined by subtracting the theoretical random variance, i.e., the mean, from the total variance of counts of the same chamber. However, the clumping error should be somewhat dependent upon the degree the sample is mixed before it is transferred to the counting chamber. The first step, then, was to determine the effect of an increased amount of mixing.

Twenty counts were, therefore, made of two different phytoplankton samples. Each count consisted of 50 fields and each field constituted 1/4750 of the slide. The first sample, PP5808, consisted almost entirely of *Chaetoceros* species while the second sample, PP6301A, was practically a pure culture of the dinoflagellate *Prorocentrum micans* Ehr.

For the first 10 counts of each series, the samples were mixed according to the routine procedure employed at the Scripps Institution of Oceanography, i.e., the mixing bottle (a ten ml. graduate cylinder) was reversed 20 times and the chamber immediately filled by placing the coverslip diagonally across the chamber and running in the sample with a pipette until the volume was just sufficient to float the coverslip into position. For the second 10 counts, the mixing bottle was shaken vigorously for one minute. This resulted in the second 10 counts receiving approximately six times as much shaking as the first 10 counts. After each count the contents of the counting chamber were poured back into the mixing bottle. At least 24 hours elapsed between each count.

To determine the effect of the two treatments, the counts were set up into an array of two groups each consisting of 10 columns of 50 fields, i.e., each count constituted a column of 50 fields, and the variance within and between the two groups determined. The amount of variance that was removed by the more thorough mixing should, then, be given by the difference between the mean squares (estimates of total variance) from within group 1 and from within group 2. The results of the calculations of these quantities are summarized in Table 1.

The mean squares of the two groups do not test significantly in either

TABLE 1. Analysis of variance for the effect of mixing.

Sample	Source of variation	Sum of squares	d.f.	Mean square
PP5808	Between groups	1.849	1	1.849
	Within group 1	1036.5	499	2.097
	Within group 2	1035.5	499	2.095
	Total	2073.9	999	
PP6301A	Between groups	14.641	1	14.641
	Within group 1	2581.2	499	5.1728
	Within group 2	2451.8	499	4.9134
	Total	5047.6	999	

series. This suggests that clumping did not contribute to the error of these counts. Final proof of this, however, will depend upon whether or not the variance of counts of the same chamber is greater than the theoretical amount. In any case the above analysis shows that a variation in the amount of mixing did not effect the total variance.

The two groups of the above analysis were then combined and an estimate of the total obtained from the variation within each chamber and compared with an estimate obtained from the variation between the means of the chambers. The first estimate will be effected by random variation plus any possible variation due to clumping while the second estimate will also include a variation due to filling errors. The results of this analysis are given in Table 2.

TABLE 2. Analysis of variance for filling error.

Sample	Source of variation	Sum of squares	d.f.	Mean square
PP5808	Within chambers	1971.1	980	2.011
	Between chambers	102.8	19	5.410**
	Total	2073.9	999	
PP6301A	Within chambers	4685.6	980	4.781
	Between chambers	362.0	19	19.05**
	Total	5047.6	999	

** Highly significant; F beyond the one per cent value.

From Table 2 it is seen that there is a significant difference between these two estimates of variance and it is evident that volume variations in filling the chamber were contributing to the total error of these counts. Upon dividing the mean square for within chambers by the mean, we obtain $2.011/2.49=0.81$ for PP5808 and $4.78/5.14=0.93$ for PP6301A. Theoretically this ratio should equal unity (or slightly less than unity because of crowding) and, since any additional source of variation would tend to raise the value of this ratio, clumping is ruled out as a source of error.

The extent of the filling error can be determined from the equation

$$\sigma_{\bar{x}}^2 = \frac{\sigma_r^2}{mk} + \frac{\sigma_f^2}{k}$$

where $\sigma_{\bar{x}}^2$ = variance of the mean,
σ_r^2 = variance associated with random distribution,
σ_f^2 = variance associated with filling the chamber,
m = number of fields counted per chamber, and
k = number of chambers counted.

In Table 2 the mean square within chambers is equal to σ_r^2 and the mean square between chambers is equal to $50\ \sigma_{\bar{x}}^2$. σ_f^2, then, is equal to 0.068 for PP5808 and 0.285 for PP6301A. It is evident from the above equation for $\sigma_{\bar{x}}^2$ that the relative effect of σ_f^2 upon the total error will vary with the number of fields and chambers counted. If we adopt the coefficient of variation as a measure of the error, we obtain for the error of the mean:

$$\text{C.V.} = \frac{100\sigma_{\bar{x}}^2}{\overline{X}} = \frac{100\left(\frac{\sigma_r^2}{mk} + \frac{\sigma_f^2}{k}\right)^{1/2}}{\overline{X}}$$

and if we assume a Poisson distribution,

$$\text{C.V.} = 100\left(\frac{1}{mk\overline{X}} + \frac{\sigma_f^2}{k\overline{X}^2}\right)^{1/2}.$$

Now

$$\sigma_f^2 = \frac{\sum\left[\frac{\overline{X}}{\overline{V}}(V_j - \overline{V})\right]^2}{k-1} = \frac{\overline{X}^2}{\overline{V}^2}\sigma_v^2$$

where V_j is the volume of the sample in chamber j, $\overline{V}$ is the mean volume, and σ_v^2 is the variance of the volume of the sample taken. The coefficient of variation then simplifies to

$$100\left(\frac{1}{mk\overline{X}} + \frac{\sigma_v^2}{\overline{V}}\right)^{1/2}.$$

In this form it is seen that the error due to filling variations varies only with the square root of the number of chambers counted in contrast to the error due to random variation which varies with the square root of the number of occurrences counted. From the values of σ_f^2 and the means of the two series of counts and by assuming V to be equal to 1 ml., σ_v^2 is found to be 0.011 ml. and 0.008 ml. for samples PP5898 and PP6301A, respectively. Substituting in the mean of these two values, we obtain:

$$\text{C.V.} = 100\left(\frac{1}{mk\overline{X}} + \frac{0.0109}{k}\right)^{1/2}.$$

From this form we can determine the effect of the filling error upon the total error.

Table 3 shows that the variation associated with filling the chamber caused an appreciable increase in the total error. Accordingly, the procedure of filling the Sedgwick-Rafter chamber was changed in that a 1 ml. bacterological pipette was used to transfer exactly 1 ml. of material from the mixing vial to the chamber.

Table 3. Effect of filling error upon total error.

No. of occurrences counted	C.V. with both sources of error per cent	C.V. with filling error removed per cent	% the error is increased by the filling error per cent
25	22.6	20.0	13.0
50	17.6	14.1	24.8
100	14.5	10.0	45.0
200	12.6	7.2	75.0

Five series of duplicate counts were then made using this method of filling the chamber. Samples PP5404 and PP4577 were dominated by *Chaetoceros* sp., PP5561 by *Skeletonema costatum* (Grev.) Cl., PP5576 by *Leptocylindrus danicus* Cl., and PP6301B by *Prorocentrum micans* Ehr. The results of these counts are summarized in Tables 4 and 5.

None of the mean squares of Table 4 tests significantly. This shows that

Table 4. Analysis of variance for filling variation.

Sample	Source of variation	Sum of squares	d.f.	Mean square
PP5004	Within chambers	869.10	490	1.373
	Between chambers	12.01	9	1.333
	Total	881.11	499	
PP4577	Within chambers	771.72	490	1.576
	Between chambers	10.33	9	1.147
	Total	782.05	499	
PP5561	Within chambers	402.06	490	0.821
	Between chambers	5.69	9	0.632
	Total	407.75	499	
PP5576	Within chambers	615.04	490	1.256
	Between chambers	7.66	9	0.851
	Total	622.70	499	
PP6301B	Within chambers	1151.18	490	2.349
	Between chambers	23.13	9	2.570
	Total	1174.31	499	

TABLE 5. Results of five series of repeated counts.

Sample	Fraction of slide covered by one field	$\overline{X}$	s^2	$s^2/\overline{X}$
PP5004	1/28900	1.878	1.773	0.94
PP4577	1/28900	1.648	1.576	0.96
PP5561	1/28900	0.850	0.821	0.97
PP5576	1/28900	1.314	1.256	0.96
PP6301B	1/6870	2.376	2.349	0.99

the filling error had been completely eliminated by the use of the transfer pipette and that all of the error of these counts could be accounted for by random distribution. In Table 5 the column of values for $s^2/\overline{X}$ indicates that the distribution in the counting chamber of these organisms followed the Poisson distribution. This is proven more rigorously by evaluating $ns^2/\overline{X}$ as chi-square with n degrees of freedom. The variances of each chamber were so evaluated and the appropriate probability found for the summation value of the chi-squares for the ten counts of each series (cf., Berkson, 1940). As shown in Table 6 all the probabilities were above the five per cent level of significance.

TABLE 6. Variance ratio test for "goodness of fit" Poisson distribution.

Sample	X^2	d.f.	P
PP5004	434.9	490	0.07
PP4577	462.5	490	0.38
PP5561	468.6	490	0.50
PP5576	464.7	490	0.43
PP6301B	475.1	490	0.64

When dealing with occurrences, therefore, we can use the simple relationship $\text{C.V.} = 100/\sqrt{N}$, where N is the total number of occurrences counted, to measure the total error and to determine how large a fraction of the slide must be counted to achieve a chosen degree of reliability.

The theoretical treatment of the random variation of total cells is, as previously mentioned, greatly complicated by the presence of colonial forms. Here we are really dealing with the product of two frequency distributions; namely, the Poisson distribution of occurrences and an unknown and variable distribution of the number of cells per colony. The relation of these two frequencies is further complicated by the probability of a varying amount of correlation between the number of occurrences and the number of cells per occurrence, i.e., one can expect that the environmental conditions that bring about an increase in the number of occurrences will in many cases also be responsible for an increase in the number of cells per colony but that the relative increases will vary with the components and past his-

tory of the plankton. Experimental suggestion that this correlation does exist is found in the fact that the variance of total cells calculated from the equation $\sigma_{xy}^2 = \sigma_x^2\sigma_y^2 + \overline{X}^2\sigma_y^2 + \overline{Y}\sigma_x^2$, which is based upon the assumption that there is no correlation between x and y, is invariably higher than the actual variance.

It is doubtful if any single theoretical frequency distribution can be found that will approximate the distributions actually encountered and at the same time furnish a practical means of approximating the variance of this material. The difficulty is most readily seen if the distribution of total cells is considered to be the sum of the Poisson distributions of the different sized colonies. It can easily be shown (cf., Lüders, 1935) that the probability function for events occurring in groups of one's, two's, three's, etc., where the groups themselves are distributed according to the Poisson distribution is:

$$P_n = e^{-h_1-h_2-\cdots-h_n} \sum_{x_1+2x_2+\cdots+nx_n=n} \frac{h_1^{x_1} \cdot h_2^{x_2} \cdot \cdots \cdot h_n^{x_n}}{x_1!\ x_2! \cdots x_n!}$$

where h_1 = mean of single occurring events,
h_2 = mean of double occurring events,
.
.
.
h_n = mean of n fold occurring events,
and x_1 = no. of single events occurring,
x_2 = no. of double events occurring,
.
.
.
x_n = no. of n fold events occurring.

The first moment or mean of this distribution is equal to $x_1+2x_2+3x_3 + \cdots + nx_n$. The second moment or the variance is equal to $x_1+2^2x_2 +3^2x_3+ \cdots + n^2x_n$. From the second moment it is clearly evident that the variance will be exceedingly sensitive to the range in size of the colonial forms. One colony of 25 cells, for example, would contribute as much to the variance as would 625 non-colonial forms. This size distribution of colonies, being dependent upon the kinds and condition of the plankton elements of any particular population, is entirely unpredictable. There is no assurance at all that a frequency distribution of colony size determined from any number of samples would approximate the distribution in the next sample studied.

The best that one can do under these circumstances is to empirically determine the ratio between the error of total cells and the error of occurrences in several extreme cases and use this relationship to roughly

approximate the maximum error of other samples. This approximation will be too high in practically every case but such a margin of safety is necessary since there is no probability function for this ratio.

Of the seven samples studied in this paper, all except PP6301A and PP6301B were composed to a large extent of colonial forms and had been chosen because of their extreme colonial composition. They can, therefore, be used to determine the ratio between the error of total cells and the error of occurrences. The coefficient of variation of total cells, just as with occurrences, will vary with the sample size. While we do not know the exact relation, we do know from the above equation for σ_{xy}^2 that the coefficient of variation is a function of the number of occurrences counted. For this reason the coefficients were calculated upon the basis of the same number of occurrences. The number of occurrences chosen will not effect the value of the ratio as long as the coefficients of all the samples are calculated upon the same basis. Using primes to distinguish the statistics of total cells from those of occurrences and choosing 100 as our reference number of occurrences, we can cast the formula for the coefficient of variation into the following form:

$$\text{C.V.} = \frac{100\,\sigma_x'}{\overline{X}'} = \frac{100\sqrt{\frac{\overline{\sigma_x'^2}}{m}}}{\overline{X}'} = \frac{100\sqrt{\frac{\overline{X}\,\overline{\sigma_x'^2}}{100}}}{\overline{X}'},$$

where m is the number of fields that would have to be examined in order to count 100 occurrences. The coefficients, calculated from the above formula, are given in Table 7.

TABLE 7. C.V.'s for total cells

Sample	$\overline{X}$	s'^2	$\overline{X}'$	C.V.' per cent
PP5808	2.49	68.1	10.10	12.9
PP5004	1.88	65.6	8.56	13.0
PP4577	1.65	23.0	4.93	12.5
PP5561	0.85	77.3	6.36	12.7
PP5576	1.31	15.1	3.73	11.9
PP6301B	2.38	2.4	2.38	10.0

Since the coefficient of variation for 100 occurrences is 10 per cent, the maximum value obtained for the ratio between the error of total cells and the error of occurrences is 1.3. In application, then, the coefficient of variation for occurrences would be multiplied by 1.3 to obtain a maximum estimate of the coefficient of total cells, i.e., $\text{C.V.} = 130/\sqrt{N}$. Or from another point of view, $(130)^2/(\text{C.V.})^2$ occurrences must be counted to be certain of an error in total cells equal to or less than C.V.

This is admittedly a crude approximation of the total error but at the same time the surprisingly small difference between the error of total cells and the error of occurrences shows that the approximation is accurate enough for many practical purposes. The error of the approximation will practically always be less than 30 per cent and can only operate so as to increase the confidence in the estimate.

I wish to express my appreciation to Prof. W. E. Allen, at whose suggestion the paper was prepared, for his continued interest and aid; to Mr. R. D. Gordon for much invaluable assistance with statistical theory; and to Dr. C. E. ZoBell and Dr. H. U. Svedrup for aid in revising the manuscript.

Summary

1. The error made in enumerating phytoplankton by means of the Sedgwick-Rafter chamber was determined and separated into its component errors.

2. Clumping of occurrences was not found to be a source of error in the samples studied.

3. After an error due to variations in the volume of sample placed in the chamber had been eliminated, the variance of these counts was found to be wholly accounted for by random distribution.

4. The distribution of occurrences, i.e., colonies and non-colonial cells, was shown to closely approximate the Poisson distribution.

5. The frequency distribution of counts of total cells of phytoplankton was shown to be unpredictable.

6. The maximum empirical ratio between the error of occurrences and the error of total cells was found to be 1.3 and the suggestion was made that this ratio could be used to estimate the error of total cells and the size of the sample that should be counted. The coefficient of variation for total cells would then be given by the formula: $C.V. = 130/\sqrt{N}$, where N is the total number of occurrences counted. It follows from this formula that the number of occurrences counted should be the same for all samples.

References

Berkson, J. 1940. A note on the chi-square test, the Poisson and the binomial. Jour. Am. Statist. Assoc. 35: 362–369.

Littleford, R. A., Newcombe, C. L., and Shepherd, B. B. 1940. An experimental study of certain quantitative plankton methods. Ecology, 21: 309–322.

Lüders, R. 1934. Die Statistik der seltenen Ereignisse. Biometrika, 26: 108–128.

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WHERE DOES ADAPTATION COME IN?[1]

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To those—possibly imaginary—persons whose genetic interests are restricted to the mechanism of hereditary transmission, the problem of adaptation can have no great appeal, even if, for them, it exists at all. To others, again, whose sole interest in the organism is to analyze it as a physico-chemical machine, the question of the historical origin of this machine would seem to be a matter of quite minor importance. Some, indeed, have thought it reasonable to reject the whole notion of adaptation as anthropomorphic. In a symposium on this subject held by the American Society of Naturalists in 1912, one prominent participant expressed a view-point which, I doubt not, would still meet with approval in some quarters. This speaker relegated the conception of adaptation to the realm of the children's diseases of science—something which we would outgrow with advancing maturity. "It seems to be partly because biological problems are too complex for ready analysis at present," he tells us, "that the adaptational properties of living things are so often stated in terms other than those of the fundamental concepts of matter and energy."

Can you visualize a biological laboratory of the future in which everything observed by the investigator is to be "stated in terms of the fundamental concepts of matter and energy"? To many of us such a laboratory would

[1] Presented at a symposium on "The Genetic Basis of Evolution," before the Western Society of Naturalists, at Stanford University, December 30, 1941. (Contributions from the Scripps Institution of Oceanography, New Series No. 172.)

be a very dull place indeed, and this even to those of us who recognize no element besides matter and energy as underlying biological phenomena.

There appear to be some biologists to whom "anthropomorphism" is the object of as great a phobia as was Soviet Russia to our more conservative fellow-citizens *before* the present realignment of forces in Europe. Anthropomorphic language is quite legitimate when we use it figuratively to avoid clumsy circumlocutions. It is vastly simpler, as well as vastly more sensible, to say that a bird, in the spring, builds her nest in order to raise her brood than it is to state that she goes through the process of nest-building by means of a series of catenary reflexes, initiated by gonadial hormones, and that the structure produced by her labors, if we may judge by previously observed cases, will probably be occupied by eggs which will develop, under proper temperature conditions, into young birds. We must insist, too, that the biologist who employs the former mode of expression is not in the least fooled by the words he uses. And if he adds that the bird has evolved "instincts" which are "adapted" to perpetuating the life of the species, surely no one but a pedant can object. We may grant that the situation so described requires further analysis.

Strictly speaking, every organism is adapted to the environment in which it lives; otherwise it would not be alive. In that sense, adaptation is coextensive with life, and the problem of the origin of adaptations is as inclusive as that of the evolution of life. More usually, however, the term is applied to cases in which adjustments of the organism to more specific features of the environment are discernible. These cases are particularly striking when the organism in question has adopted an environment quite different from that of its nearer relatives or its supposed ancestors. Here we may find obvious alterations of the original pattern which seem to bear very direct relations to needs imposed by the changed conditions of life. The fact that mistaken, even absurd, guesses have been made respecting the functional value of

some of the characters of organisms is no ground for dismissing the whole subject of adaptation as a topic for idle speculation.

I have, during much of my life, been particularly interested in adaptations relating to the concealment of animals from enemies or prey. This is preeminently a field in which both unbridled imagination and dogmatic denials have been much in evidence. But it is also a field in which some of the most striking and well-authenticated examples of adaptation are to be found. Doubt of the significance of these phenomena may take two forms. The most radical degree of skepticism is shown by those few who question the protective value of these color characters altogether, and strive to find for them a purely physical or chemical interpretation. Critics of a less skeptical turn of mind are ready to admit that at times an animal's color-scheme may be of value for protective or aggressive purposes, but they challenge us to show that such characters have been developed through natural selection because they confer these advantages upon the animal. The characters in question, they say, have a cause quite independent of the animal's need for concealment. The resemblance to surrounding objects is purely incidental.

It is curious that unfounded assertions such as these still continue to pass as arguments, although their fallacy has long been clearly evident to those familiar with the facts. I have devoted considerable space in several earlier papers to a refutation of some of these contentions,[2] and will discuss only a few points here.

If we had to do simply with a general color resemblance between an animal and its more familiar background, we might invoke some possible—even though utterly unproved—direct physical influence of this background upon the animal's skin. Thus we should be able to talk, as we are told a biologist should talk, "in terms of the fundamental concepts of matter and energy."

When, however, as often happens, an animal distributes

[2] AMERICAN NATURALIST, Vol. 69: 1935; *Proceedings of the National Academy of Sciences*, Vol. 20, 1934, and Vol. 21, 1935.

its pigment in significant patterns on the body surface and develops appendages on the skin, thus closely matching details of its usual habitat, another kind of vocabulary seems called for. Most of all, when the animal acquires a mechanism for color-change which is almost coextensive with the entire organism, involving as it does the eyes, large parts of the nervous system, highly specialized effector cells in the skin, and frequently the secretions of endocrine glands, and when, too, no function seems conceivable except that of rendering the organism inconspicuous against backgrounds of varying color and pattern, we seem forced to resort to a purely biological vocabulary and talk in terms of concealment from enemies or prey. I can not take time to discuss the experimental evidence which exists for the truth of this almost self-evident interpretation of the phenomena. I will only say that some of this evidence seems to me to be decisive.

Important light is being thrown upon both the physics and the chemistry of these processes of color change in animals, and we may hope that, in time, a fairly complete account of these may be written. Let us insist, however, that such an account will in no sense supersede, but will merely supplement, the biological account of these phenomena, which is a correct one so far as it goes.

How are we to explain the existence of such a mechanism as this on the basis of any one of the familiar evolutionary theories?

The Lamarckian hypothesis would explain it if this were otherwise admissible. The organism, according to this hypothesis, has striven to bring its appearance into harmony with that of its background. The results of each effort in that direction have been inherited in some measure by its offspring, and thus, in the course of many generations, the present marvelously efficient mechanism has been developed. This is a valid scientific hypothesis with no necessary taint of mysticism about it. It is an explanation, moreover, which has much *prima facie* probability. For it is a fact that many organisms can and do change their colors under the influence of the sense organs

and nervous system. (Whether we credit these animals with *effort* is a matter of small consequence.) Unfortunately, there is one serious, possibly fatal, obstacle in the way of accepting this type of explanation. I do not refer to our present inability to conceive of a probable mechanism through which the effects of functional activity can be inherited. That is not a fatal obstacle at all. I refer to the seeming total lack of convincing evidence that such a mode of inheritance exists. This last is a fact of much weight when we consider the intensive search for such evidence which has been carried on for many years. For the present, we seem forced to disregard Lamarckism as an explanation of the origin of adaptive mechanisms.

Thus, unless we are disposed to flirt with mysticism, or to accept purely verbal explanations of natural phenomena, there would seem left to us only one evolutionary hypothesis worth considering, that of natural selection. However, when we have said that, we have left some of the most important things unsaid. For we may, on the one hand, conceive of natural selection as acting continuously throughout the evolutionary process and accumulating small variations in the direction of fitness; or we may conceive of it merely as setting its stamp of approval upon a finished product. In the latter case, the role of natural selection is greatly restricted, and we must look elsewhere for the origin of adaptations. But if Lamarckism be ruled out, where shall we look? We all agree, I trust, that "inner perfecting tendencies" and the like do not belong in the realm of science.

Many of us had probably regarded the hypothesis of evolution through large, abrupt steps as having been finally disposed of. But its recent revival by one of our leading geneticists warrants giving it some serious attention. The subject has been discussed by many able biologists from Darwin down, and I shall merely review some of the considerations which seem to me to render this type of theory unacceptable.

My present discussion, let me repeat, relates to evolution through large, abrupt steps. Of course the query

arises forthwith: How large is "large"? Goldschmidt makes it plain that he has in mind the abrupt origin of the major categories of classification. He cites, at least without protest, the repetition by a recent paleontologist of St. Hilaire's well-known suggestion that the first bird may have emerged from the egg of a reptile. It may be recalled that the Dutch botanist Lotsy put forth, in 1916, somewhat similar views regarding the origin of the main subdivisions of the living world, offering as an example the probable sudden appearance of the vertebrate phylum through the hybridization of two invertebrates.

Such assumptions certainly avoid some of the difficulties inherent in the theory of selection as contemplated by Darwin, particularly the embarrassing scarcity of connecting links between major taxonomic groups, and the improbability that selection can act on the "pre-useful stages" of many organic structures. If none of these connecting links ever existed, we don't have to expect our paleontologists to find them; and if our main adaptive structures came into existence full-fledged and ready for use, we don't need to worry about pre-useful stages. These are such obvious advantages in the "saltatory" types of evolutionary theory that their proponents do not seem to be sufficiently impressed by their difficulties. But just in proportion as we conceive evolutional steps to be great enough to avoid the difficulties just mentioned, are we forced to throw an impossible burden upon chance.

I don't know whether it is permissible, in the present advanced state of our knowledge, to quote such a back-number as Charles Darwin, but my temptation in this case is strong. "He who believes that some ancient form was transformed suddenly through an internal force or tendency into, for instance, one furnished with wings, will be almost compelled to assume, in opposition to all analogy, that many individuals varied simultaneously. . . . He will further be compelled to believe that many structures beautifully adapted to all the other parts of the same creature and to the surrounding conditions have been suddenly produced; and of such complex and wonderful co-

adaptations, he will not be able to assign a shadow of an explanation. . . . To admit all this is, as it seems to me, to enter into the realms of miracle, and to leave those of Science." Thus spoke Darwin!

To the saltatory evolutionist, our newly acquired lung or wing or plumage is supposed to have arisen, as a functioning entity, through a single "macromutation." In so far as he splits up these changes into smaller steps and envisages a cumulative process, he is departing from his original thesis and losing some of the advantages gained by his special type of theory. Bear in mind that none of these new acquisitions is merely a homogeneous mass of living matter, to be influenced in a "more" or "less" direction by mutation. Each is a complex, integrated structure, the success of which is altogether dependent upon the simultaneous modification of a large variety of tissues. Add to this the necessary coincident modification of other parts of the organism already present. Indeed, where we have to do with a radical change in the animal's life, as from an aquatic to a terrestrial mode of existence, there may be hardly a single part of the body which will not need revision.

Those who have perused the writings of Herbert Spencer—if any such persons remain—will recall that the existence on every hand of these "coadaptations" of structure and of function furnished him with one of his strongest lines of argument for the Lamarckian factor in evolution. It must be admitted that this hypothesis, if otherwise tenable, would provide us with a beautifully simple explanation of the facts.

Spencer's argument for the "inadequacy of natural selection," in this case, rested upon the improbability that the necessary variations in all these functionally related parts would occur simultaneously. I think we must admit that this argument is quite valid if we have in mind variations large enough to bring about considerable changes in the bodily machinery. If, on the other hand, we have in mind variations of small magnitude, of a size comparable with many of the mutations best known to

modern geneticists, the progressive variations in the several organ systems would not have to occur simultaneously. This was pointed out by Wallace in a reply to Spencer. The wide range of mutual adjustability of the various parts of the body during development would prevent serious disharmonies. Selection must be supposed to act upon the entire organism, not upon single characters. (Least of all—to bring our discussion up to date—does it commonly act upon single genes.) One individual doubtless profits by one set of advantages, another by another. All these characters, in a freely interbreeding population, may, however, be ultimately incorporated into the heritage of the species.

It seems obvious that any one who sponsors a theory of evolution by large steps must provide an agency through which functionally related parts are brought to vary together in such a way as to insure the harmonious cooperation of the parts affected. That neither genetic linkage nor the manifold effects of single genes affords a mechanism for such correlation, to more than a very limited extent, will probably be readily granted. It is probable that such an agency, if it exists, must be sought in the field of developmental physiology rather than that of genetics.

In his important recent volume on "The Material Basis of Evolution," Dr. Goldschmidt has discussed at considerable length various aspects of the physiology of growth and development. He stresses such facts as the changes in the relative size of parts which frequently result from a general increase or decrease of body size and the radical effects upon body structure of accelerations of the rate of growth. Of some of these cases, he writes that "a simple shift in the velocity of one of the integrating processes relative to the others will account for the primary change with all the later unavoidable consequences during subsequent development." Much is made by Goldschmidt of the effects of such drugs as thyroxin which hasten metamorphosis and thus give the appearance of initiating profound morphological and physiolog-

ical changes. "Feeding with thyroid makes the axolotl transform into the *Ambystoma,* a transformation which involves an immense morphogenetic change from gills to lungs, with all the concomitant changes in all systems of the body." On the other hand, the permanently gilled Perennibranchiata remain throughout life in the axolotl stage and experiment has shown that they can not be made to metamorphose: "they are genetically unfit for complete metamorphosis."

Goldschmidt puts aside as irrelevant the "problem as to whether the perennibranchiates are phylogenetically primitive or whether they are derived as neotenic larval forms from metamorphosing Amphibia. The point of our argumentation," he says, "is independent of such speculations. The facts demonstrate that there are Amphibia in which a hereditary norm of reaction permits metamorphosis, which is a morphological change of an order of magnitude characteristic of the taxonomic difference between families or orders."

I have quoted Professor Goldschmidt at some length because it seems to me that these passages illustrate a fundamental fallacy in his evolutionary argument. From the standpoint of evolution, there is really a vast difference between the induction of metamorphosis in a secondarily larval form like the adult axolotl, whose ancestors undoubtedly lost their gills at maturity, and the induction of similar far-reaching changes in forms whose ancestors may never have led a terrestrial existence. That attempts to effect such transformations in the latter group of amphibia have failed is not surprising. If they had succeeded, should we not merely have rather strong evidence that they too are neotenic larvae like the axolotl?

Goldschmidt has surely marshalled a highly interesting set of facts along the lines indicated. But before accepting his deductions from these facts, we must await the discovery of a case which he has not yet presented. This would be a case in which the hormonally induced remodelling of an organism has resulted in the appearance of a set of adaptive structures new to its ancestral his-

tory. It is easy to demonstrate that a single genetic change may bring about the total loss of a number of complex structures. But this throws no light whatever on the processes through which these structures arose in evolutionary history. A person seeking information on the manufacture of watches would not be much enlightened by learning that one of these instruments could be demolished by a single blow of a hammer. As little can we draw valid conclusions concerning racial history from the experimental restoration of a structure formerly present but now suppressed under the usual conditions of life.

If we admit the preceding contentions, we must also admit that all these interesting facts relating to norms of reactivity, seasonal dimorphism, endocrine control, homoeosis, neotenic larvae, rudimentation, etc., while vastly important as contributions to our knowledge of ontogeny, have little bearing upon the mechanism of evolution.

In the present state of our knowledge, we seem driven to the rather banal conclusion that evolutionary change has resulted mainly from the accumulation through selection of small genetic differences. This is a return to Darwinism in the narrower sense of that word, though such an avowal does not imply the admission that nothing has been learned in this field since Darwin's time.

Where adaptations are of a very general sort, such as increased fecundity, resistance to disease or ability to withstand the rigors of climate, there is little difficulty in conceiving of the operations of the evolutionary process. It is but natural that recent geneticists, in citing hypothetical cases in which one mutant type is supposed to have an advantage over another, appear to have had in mind adaptations of this general type.

That some character of dubious value to the organism may be genetically correlated with an elementary advantage, say fecundity, and thus be carried along like a rider on an appropriation bill, is no novel idea, as some may have supposed, but was suggested by Darwin himself in the "Origin." It is perhaps characters of the "rider"

type which chiefly distinguish subspecies from one another. It is difficult to imagine specific adaptation to local conditions in the differing lengths of the bodily appendages of mammals and birds inhabiting different geographic areas. Or perhaps, as some have averred, adaptation has played no part, even indirectly, in the origin of such characters. It may be that the mutant genes involved in their production have gained local ascendancy through the vicissitudes of chance, operating in small populations.

At the opposite pole from the simple adaptational characters last discussed are those complex mechanisms involving the cooperation of a number of important organ systems. Here belong, for example, such cases as the mechanism of color change to which I referred at some length earlier. To Darwin, the processes by which such complicated machinery came into being were simply an extension of those which led to the minor differences between one species or variety and another, namely, variation, inheritance and the differential survival of strains displaying the advantageous characters in a higher degree.

Any such passage in evolutionary history is conceivable, as already insisted, only if we recognize its gradualness. To begin with, this adaptive mechanism did not arise *ex nihilo*. It was built upon an antecedent foundation of nervous and other elements acquired early in the ancestral history of the vertebrate stock. And throughout the entire period of its development, this faculty must have shown quantitative differences in its degree of perfection. That individual differences in the capacity for chromatic adaptation exist between members of the same species is a familiar fact to those who have experimented in this field. It may be fairly assumed, too, that these differences are in part genetic. Presumably multiple factors have been concerned in a large way. On the basis of all analogy, this is a faculty which we should be able to improve greatly by means of artificial selection. That

natural selection has been operative in the present case is rendered doubly probable by experimental proof that the faculty in question may be of life-saving importance.

Unfortunately, acceptance of this account of the origin of adaptive characters through natural selection has been retarded by the prevalence of inadequate notions regarding the role played by mutation. Much has been said of the occasional appearance of mutant genes which give to their possessors some particular advantage. Such advantageous genes have been presumed to be gradually accumulated through selection until they become established in the germplasm of the species. Mutation and selection go hand-in-hand, it would seem, in every evolutionary advance.

In reply to this, as it seems to me misleading, account of the case, I will quote from an earlier discussion which I published some years ago. Let me repeat my disclaimer of any originality in the views here expressed.

> It is hardly necessary to point out that selection is not concerned, directly, with single genes, but with the aggregate fitness of entire organisms, and that this fitness commonly depends upon a large number of varying genetic elements. Unlike the more or less pure cultures which are frequently reared in the laboratory, natural populations are genetically highly mixed. This condition is maintained through the free interbreeding of diverse strains, probably, in extreme cases, through the crossing of widely different races or even species. Thus, while every one of the underlying unitary differences may be supposed to have originated, at some time, as a single mutation, the material upon which selection acts has arisen through the endless shuffling of these elements as a result of sexual reproduction. The processes of mutation and selection thus need not go on *pari passu*. Much genetic change might come about, and be widely disseminated throughout a population, before it became subject to selection at all. Large genetic differences might thus be available at the very commencement of a given selective process, and the gap between the "pre-useful" and the "useful" stages of a structure might be bridged by purely fortuitous gametic combinations.[3]

In writing those words, I had in mind no such large-scale remodeling of organic structure as would be called for by a truly saltatory theory of evolution. I had in mind such truth as may deservedly be credited to the notion of "pre-adaptation."

[3] Bibliographia Genetica, Vol. 9, 1932.

Contribution No. 173

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BACTERIAL ACTIVITY IN DILUTE NUTRIENT SOLUTIONS[1]

MOST media designed for the growth of heterotrophic bacteria contain from 0.1 to 1.0 per cent. of organic matter, and it is generally claimed[2] that the minimum concentration required for their multiplication ranges between 0.001 and 0.01 per cent., or between 10 and 100 mgm/l. This is considerably higher than the concentration of nutrients generally found in nature in lakes, soil solutions or sea water. Sea water contains an average of 4 or 5 mgm/l of organic matter, much of which is fairly refractory to bacterial decomposition,[3] yet there is evidence that bacteria multiply and are otherwise active in sea water. In fact, the bacterial population may increase from a few hundred bacteria per ml of freshly collected sea water to several million after a few days' incubation in the laboratory.[4]

In order to approach the minimum quantity of organic matter which limits bacterial activity, an organic matter-free mineral solution was prepared. To it was added enough peptone to give concentrations ranging from 1.0 to 100 mgm/l, after which test-tube quantities were inoculated with a loopful of organic matter-free water containing from 10 to 100 living bacterial cells. After thoroughly mixing, loopful quantities were streaked on nutrient agar plates and the procedure was repeated at intervals of 24 hours. So few cells were introduced that rarely did any growth occur on the plates inoculated initially. However, after 24 hours' incubation at 22° C ten out of twelve of the cultures tested had multiplied enough to produce an abundant growth on the nutrient agar when loopful quantities were transferred. Since the controls were properly negative, the experiment showed that the cultures had multiplied in the most

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 173.

[2] Marjory Stephenson, "Bacterial Metabolism," Longmans, Green and Company, 1939.

[3] S. A. Waksman and C. L. Carey, *Jour. Bact.*, 29: 545, 1935.

[4] C. E. ZoBell and D. Q. Anderson, *Biol. Bull.*, 71: 324, 1936.

dilute of the peptone mineral solutions, although only the solutions containing more than 10 mgm/l of peptone were turbid. Similar results were obtained in dilute glucose ammoniacal mineral solutions.

It is not surprising that the dilute nutrient solutions do not become cloudy with bacterial growth because it requires around a billion cells per ml of the size of those being used to produce perceptible turbidity. Even if all the organic matter (1 to 10 mgm/l) were assimilated, there wouldn't be enough to give the requisite number of cells to produce a turbid solution. Moreover, many of the cells in dilute nutrient solutions grow attached to the walls of the test-tube.[5]

Quantitative results were obtained by inoculating glass-stoppered bottles filled with mineral solution treated with concentrations of glucose, ranging from 0.1 to 10 mgm/l. After different periods of time the bacterial populations were determined by plate count procedures and the dissolved oxygen content of the water was determined. The results showed that the bacteria multiplied in concentrations of glucose as low as 0.1 mgm/l and that this amount of glucose was completely assimilated in four or five days at 22° C. Ten to twenty days were required for the complete assimilation of concentrations of glucose as large as 1 to 5 mgm/l. Between 60 and 70 per cent. of the glucose was oxidized and the remainder was converted into bacterial protoplasm. Similar results were obtained with glycerol, ethanol, succinic acid and lactic acid. As will be elaborated elsewhere solid surfaces seem to facilitate the assimilation of dilute nutrients. Under proper conditions it is believed that concentrations of utilizable organic matter considerably smaller than 0.1 mgm/l will provide for bacterial multiplication.

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[5] C. E. ZoBell, *Jour. Bact.*, 33: 86, 1937.

[6] On sabbatical leave from Brooklyn College, Brooklyn, N. Y. Assisted by grant No. 555 from the American Philosophical Society.

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THE RESISTANCE AND ACCLIMATIZATION OF MARINE FISHES TO TEMPERATURE CHANGES. I. EXPERIMENTS WITH GIRELLA NIGRICANS (AYRES) [1]

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Introduction

Fishes occur in nature over a wide range of temperature, from the freezing point of sea water (about −2°) to at least 37° C. (Sumner and Sargent, 1940) or higher. The range of tolerance of most species apparently does not include both of these extremes. Differences of resistance to extreme temperatures shown by different species, both under experimental conditions (Huntsman and Sparks, 1924; Huntsman, 1926; Hathaway, 1927) and in nature (Storey, 1937), have been found to correspond to differences of distribution (geographic or vertical) in relation to temperature.

Quantitative studies of acclimatization, as a factor in the resistance of fishes to high temperatures, have been undertaken repeatedly (Loeb and Wasteneys, 1912; Hathaway, 1927; Binet and Morin, 1934; Sumner and Doudoroff, 1938). Detailed studies of acclimatization to low temperatures are lacking. This oversight is not surprising. The view that low temperatures are less injurious than equally extreme high temperatures, at least when freezing is not involved, is not uncommon. Heretofore, Maurel and Lagriffe (1899a, b, c) have attempted to compare experimentally the upper and lower limits of tolerance of some, chiefly fresh-water, fishes. They concluded, "Qu'ils semblent mieux organisés pour résister aux températures extrêmes du froid qu'aux températures extrêmes de la chaleur." It is not clear what criterion was used in judging whether a given temperature was more or less "extreme" than another. Aside from the fact that the methods used were not entirely satisfactory, it appears that the above conclusion was probably based upon some faulty line of reasoning, and is not an obvious outcome of the experimental results.

In general, the sensitivity of fishes to chilling has received little attention from experimental physiologists, who have most frequently

[1] Contribution from the Scripps Institution of Oceanography, New Series, No. 174.
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used relatively cold-hardy fresh-water and estuarine marine fishes as laboratory material. The survival of fishes which had been frozen for a long time in water, or frozen solid throughout in air, has been reported by a number of early observers (for references see Borodin, 1934). This has not been confirmed by the more reliable and recent investigations, but it has been shown that some cold-hardy fishes can survive super-cooling (i.e., without freezing) to temperatures below the freezing point of their body fluids, and also brief and partial freezing of their superficial tissues (Regnard, 1895; Britton, 1924; Weigmann, 1930, 1936; Borodin, 1934; Schmidt, Platonov and Person, 1936; Luyet, 1938). The phenomenon or possibility of death after more prolonged exposure to moderately low (above-zero) temperatures has attracted much less attention. Indeed, Luyet and Gehenio (1938), in the "compendium" of their extensive review dealing with the lower limit of vital temperatures, state: "The third group, namely that of organisms which are killed at above-zero temperatures, includes only the homoiotherms and some of the higher plants." A number of observations on the death of poikilotherms from chilling are, however, on record (e.g., Weigmann, 1929).

Changes of cold-tolerance, resulting from continued exposure to high and low temperatures, have been observed in fishes by Wells (1935) and Sumner and Doudoroff (1938), but accurate quantitative studies of their magnitude were not undertaken. There is also surprisingly little evidence available for an influence of acclimatization upon susceptibility to chilling in other animals.

The term acclimatization will be used here to designate only gradual changes in the effect upon an organism of a given environmental factor, which can be produced experimentally in the individual organism by altering its environment with respect to this particular factor. Heilbrunn (1937) introduces his interesting chapter on acclimatization with a fairly comprehensive discussion of the modern meaning of the term. However, Heilbrunn cites as examples of temperature acclimatization certain seemingly adaptive differences between individuals of the same species from different latitudes, as well as seasonal variations of resistance to extreme temperatures, etc. (e.g., Hörstadius, 1925; Bodenheimer and Klein, 1930). This is not entirely consistent with his own definition. Such variations may be inherent, i.e., sub-specific (resulting from mutation and selection) or inherently periodic, or they may be related to differences of diet or to environmental differences other than those of temperature. The use of the more general term "adaptation" (Bělehrádek, 1935; Fox, 1939) is

preferable in referring to such variations, which have not been shown to be due to acclimatization alone, as defined here.

True acclimatization to cold, as indicated by changes of resistance to freezing (sub-zero temperatures), has been studied in insects by Payne (1926a, b) and by Mellanby (1939). Mellanby (1939, 1940) also investigated the influence of acclimatization upon resistance to immobilization by cold at non-freezing temperatures (chill-coma) in insects and amphibia. The influence upon survival at such temperatures was not studied in detail. Ogle and Mills (1933) produced changes of cold-tolerance in rabbits. The mechanisms of acclimatization in cases in which death is associated with a thermoregulative breakdown (in strict homoiotherms), or with the freezing of body fluids, are probably quite different from that involved in changes of resistance of poikilotherms to chilling.

The acclimatization of animals to heat is well known. Studies with fishes have already been cited. Heilbrunn (1937) cites other references.

The present paper deals with quantitative experiments on the resistance of the marine fish *Girella nigricans* to extreme temperatures and on the phenomena of acclimatization to heat and especially to cold, and with their ecological significance. Experiments with other species and a discussion of some possible causes of death at extreme low temperatures will be presented in a later publication.

I am indebted to Dr. F. B. Sumner, of the Scripps Institution of Oceanography, for his invaluable advice and unfailing interest during the progress of this investigation. Grateful acknowledgment is also given to other members of the staff of the Scripps Institution for helpful suggestions, information and assistance.

Material

The young (immature) greenfish, *Girella nigricans* (Ayres), used in the experiments were taken in the intertidal area near the Scripps Institution, La Jolla, California. This marine shore-fish is an active and fairly hardy inhabitant of the littoral zone, occurring in open water and in tide-pools along the open coast.

For proper evaluation of the results, with respect to their ecological implications, information relative to temperature conditions to which the animals are normally exposed in their natural habitat is necessary. The following data are based on daily records of water temperatures at the end of the Scripps Institution's 1000-foot pier. The average surface temperature for 23 years (1916 to 1938) was 17.0° C. The average for January and February, the coldest months, was 14.0°, and that for July and August, the warmest months, was 20.6°. The lowest

and highest monthly averages on record were 11.8° and 23.6°, respectively. Corresponding weekly averages were 11.7° and 25.1°. More significant as indices of the normal temperature range are the mean yearly minimum and maximum, that is, means of the lowest and highest temperatures, respectively, occurring during each summer and each winter. Since the daily records were not available to the writer, means of the lowest and highest weekly averages were calculated. These values were 13.3° and 22.0°. The average bottom temperature at a depth of 5 meters was 0.8° lower than at the surface. This vertical gradient averaged 1.9° in 5 meters during the two warmest months. The range of daily temperatures at a given level within a single week sometimes exceeds 5° or even 7°.

In tide-pools in which some Girella remain during low tide the temperature generally does not differ greatly from that in open water, but occasionally temperatures above 26° have been observed. According to Jordan, Evermann and Clark (1930), the range of distribution of *G. nigricans* extends along the "coast of California from Monterey to Cape San Lucas," i.e., further southward than northward from La Jolla.[3] This probably does not represent the extent of the normal habitat of the species. Since different physiological races or subspecies may be involved, experimental results obtained with specimens from one locality are not strictly applicable to those from other localities.

Methods

In comparing the resistance of fishes to extreme temperatures, some workers (e.g., Loeb and Wasteneys, 1912) determined the average duration of survival at constant lethal temperatures. Others (Maurel and Lagriffe, 1899a, b; Huntsman and Sparks, 1924; Sumner and Doudoroff, 1938; et al.) determined the temperature at which death or coma occurred when the water was warmed or cooled fairly rapidly. These methods are satisfactory for some purposes, but the results are not readily applicable to many ecological problems. The ecologist is interested in knowing and comparing the most extreme temperatures at which organisms can survive indefinitely, or at least for equal and long periods of exposure. That the limit of tolerance varies with the duration of exposure involved is well known, and one may not assume that closely parallel relations always hold between temperatures just tolerated for brief periods and ones tolerated for long periods, especially by different species.

More suitable for present purposes was the method used by Hathaway (1927) in experiments on heat-tolerance. It involved the use of

[3] For data on surface temperatures in this range see U. S. Weather Bureau (1938)

a series of constant test temperatures and fixed periods of exposure. Hathaway's calculated "average tolerance limits," do not, however, actually represent what the term implies, being lower than the true values. For any given period of exposure he regarded a given temperature as being the limit of tolerance for a percentage of the experimental animals obtained by taking the difference between the percentages surviving at this temperature and at the next higher (more extreme) test temperature. The formula used yielded the mean of the "tolerance limits" thus obtained. Yet the interval between the two test temperatures corresponds to a statistical "class-interval," into which fall the individual tolerance limits of the above percentage of specimens. The usual statistical procedure is to assume uniform distribution over the class-interval, and in computing the mean, the mid-value (not the lower limit) of each class is taken as the average value for all items in that class.

In the present experiments, instead of the mean, the *median tolerance limit* was adopted as a measure of resistance. The upper or lower median tolerance limit (T_m) for any given period of exposure will be defined as a temperature at which just 50 per cent of the specimens under consideration are able to survive for this period of time.[4] This value is more easily estimated than the mean tolerance limit, and is not affected by occasional extreme variants, such as unhealthy individuals.

The fishes were held in large tanks supplied with running sea water. Constant temperatures were maintained by thermostatic control or otherwise, and did not deviate from the stated values by more than 0.5° C. (usually less). When temperatures above 20° were maintained. the removal of excess air (supersaturation) was provided for by bubbling a stream of compressed air through the heated water before it entered the aquaria.

In testing their heat-tolerance, the fishes were transferred to an aquarium supplied with an ample flow (about one liter per minute) of heated and air-equilibrated water. The temperature was kept constant by thermostatic control and generally did not deviate from the stated values by more than 0.1°, the maximal deviation allowed being 0.2°. The same degree of accuracy was maintained in cold-tolerance tests. These were performed in two ways. In early (long-time) experiments the aquarium was supplied with cooled (but not aerated) running water. In later experiments, in which the periods of exposure were limited to 24 hours, the tests were made in about nine liters of standing water in glass battery jars immersed in a constant-

[4] Obviously, equal numbers of specimens have tolerance limits above and below this value; hence the designation "median."

temperature tank. The water in this case was well aerated with compressed air. In a given series of experiments the same method was used throughout.

The determination of the upper or lower median tolerance limit of a group of fishes for any desired period of exposure was made in the following manner. A number of specimens (usually ten or more) were transferred directly to the test tank, maintained at a temperature which was believed to be close to the limit of tolerance. At the end of the desired time interval the number of specimens surviving was noted. If exactly 50 per cent of those tested survived, this temperature was taken as the median tolerance limit. If more or less than 50 per cent survived, similar tests were performed at other temperatures 1° C. (or, in a few instances 0.5°) apart, until two temperatures were found at one of which more than half and at the other less than half of the specimens tested survived. The median tolerance limit was then estimated by graphical interpolation. The percentage surviving at each of the two temperatures was plotted against the temperature on arithmetic co-ordinate paper, and the two points connected by a straight line. The temperature which corresponded to 50 per cent survival on this graph was taken as the median tolerance limit.[5] What is meant by survival will be explained later.

The maximal error in the estimation of the median which may result from the assumption of a straight-line relationship between the experimental points is, for present purposes, negligible, not exceeding a few tenths of one degree. The differences of tolerance in which the writer was interested were so clear-cut that the computation of the probability of their significance would be superfluous.

Results

Effects of Extreme Temperatures and the Criterion of Survival Adopted

At rapidly lethal high temperatures great stimulation, loss of equilibrium, and finally permanent and almost simultaneous cessation of respiration and of other movements (heat-coma) were observed. At slowly lethal high temperatures stimulation, disturbances of equilibrium and other signs of distress (initial shock effects) were frequently observed directly after transfer from low temperatures. This was followed by temporary recovery and more or less normal behavior. Specimens conditioned to high temperatures began to show distress only a few hours or minutes before death. Thus, neither recovery from the initial effects, nor failure to show any distress after transfer to a

[5] The upper median tolerance limit is identical with the "thermal index" used by Whitney (1939) in comparing the heat-resistance of mayfly nymphs.

high temperature, is an indication that the fish will survive the change. Recovery from heat-coma after return to a normal temperature was never observed in Girella.

At lethal low temperatures the initial shock effect was generally more pronounced than at high temperatures. It ranged from mild distress and disturbance of equilibrium to a violent, convulsive paroxysm, followed by cessation of respiratory and all other movements. I shall refer to the latter state as "primary chill-coma." At very low temperatures the fishes died without showing any further visible signs of life, except, in some cases, a brief quivering of the opercles and fins. At less extreme temperatures the fishes recovered more or less from the initial shock. Regular respiratory movements, if they had ceased, were resumed, and frequently the fishes righted themselves and regained normal appearance and behavior. After some hours or days, however, they again showed increasing distress, and finally ceased to respire and to respond to stimulation. This stage, to which I shall refer as "secondary chill-coma," was soon followed by death.

The initial shock was not manifest until several seconds after transfer to the low temperature, and apparently was not due to stimulation of the cutaneous sense organs, but was produced only when the low temperature had penetrated internally, probably to the central nervous system. Accordingly, it was more delayed in large specimens than in small ones. By cooling the animals gradually the shock was sometimes apparently prevented or reduced without appreciably delaying the onset of secondary chill-coma.

If it occurred at all, spontaneous temporary recovery from primary chill-coma (i.e., resumption of respiratory and other movements, occurring at the low temperature at which the coma was produced) began within a few minutes or, at most, within half an hour. Thus, in Girella primary chill-coma was not a very pronounced phenomenon, except at very rapidly lethal temperatures. In most cases the initial shock effect, although intense, did not involve complete coma of appreciable duration. In other species, however, primary chill-coma, followed by temporary recovery, may be quite prolonged. If returned to a normal temperature within one hour, Girella in a state of primary chill-coma were frequently capable of rapid and permanent recovery. After the onset of secondary chill-coma, spontaneous recovery was never observed, but the fishes sometimes showed brief and partial or even complete recovery on warming, provided that they were returned to the normal temperature within a few minutes. The more slowly lethal the temperature and, accordingly, the more delayed the onset of

secondary coma, the less likely were the fishes to recover on warming. The administration of artificial respiration, by passing a stream of water through the mouth and over the gills, favored recovery.

The accurate quantitative comparison of relative tolerances requires the adoption of a uniform and convenient "criterion of survival," that is an end-point after which the organism is regarded as having succumbed to the effects of the lethal agent. It is best to adopt as an end-point the permanent (not spontaneously reversible) cessation of some important function which is among the first to be arrested. The time required for the attainment of an advanced stage of necrobiosis is not as accurately indicative of relative rates of injury, and may be greatly influenced by temperature and other factors independently of the rate of injury. The permanent cessation of respiratory and other movements, either spontaneous or induced by mechanical stimulation, presumably resulting from the functional inactivation of the central nervous system, apparently satisfies the above requirements.

In the determination of heat-tolerance the application of the end-point adopted (i.e., cessation of all spontaneous and reflex movements) presented no difficulty. The occurrence of two types of chill-coma introduced some difficulty. Primary chill-coma differs from chill-coma which has been described in insects (Mellanby, 1939) and other animals in that the fishes were capable of rapid adaptation to the low temperature, with consequent temporary recovery. In this respect it differs also from heat-coma. On the other hand, the onset of secondary chill-coma is comparable with that of heat-coma and a satisfactory end-point. Neither was ever followed by spontaneous recovery, and both apparently represented the onset of death, as the fishes failed to recover on return to normal temperatures or lost the ability to do so within a very short time. At very extreme low temperatures, at which no spontaneous recovery from primary coma was noted, an entirely comparable end-point was lacking. In practice, Girella were regarded as having succumbed if they had failed to resume movements at the end of one hour after transfer. Thereafter, as after the onset of secondary coma, spontaneous recovery was apparently impossible, and recovery on warming was only rarely observed.

By way of summary, it can be stated that a specimen was regarded as having succumbed if no movements, either spontaneous or induced by mechanical stimulation, could be detected, but no determinations of lower tolerance limits were made for periods of exposure shorter than one hour. In this way any uncertainty as to whether movements had ceased permanently at the time of observation or had been suspended temporarily was eliminated.

Time-Temperature Relations of Heat and Cold Tolerance and the Influence of Acclimatization

The specimens used in the first series of quantitative experiments were collected in the fall of 1939, and held at 20° for three and a half months.[6] On March 14, 1940, they were divided into three lots, which were acclimatized thereafter to 12°, 20° and 28°, respectively, for nearly two months before tolerance determinations were begun. Evidence to be presented later indicates that the fishes were then completely acclimatized to the new temperatures, that is, their tolerance had reached a constant value and would not change appreciably with more prolonged acclimatization. Therefore, the results of subsequent tests may be regarded as comparable, in spite of unavoidable differences in the duration of acclimatization previous to testing.

Groups of eight to 14 specimens of similar history were tested in running water at a series of low or high temperatures, usually 1° C. apart. The number surviving was recorded at fixed intervals of time, namely, after 1, 3, 6, 12, 24, 48 and 72 hours of exposure to the test temperatures, and sometimes also after other periods.[7] The lengths of the specimens ranged from 6.0 cm. to 9.2 cm. The mean lengths in the different groups under comparison were approximately equal, although those of lots tested at high temperatures were slightly greater than those of lots tested at low temperatures. Since no obvious correlation was noted between the duration of survival and the size of the individual specimens, the smaller differences between the mean lengths of the test lots could be of no great consequence.

The experimental data are presented in Tables I and II. From these data the lower and upper median tolerance limits for the various periods of exposure after which observations were made were estimated by the method described earlier and plotted against the duration of exposure in Figures 1 and 2. The resulting curves will be referred to as the time-temperature curves of cold-tolerance and of heat-tolerance, respectively.

From Tables I and II it may be deduced that among specimens of similar history the variability of the individual tolerance limits (i.e., of the lowest and highest temperatures which individual specimens are

[6] The appearance in the stock-tank of a fatal, seemingly infectious disease (dermatitis), which was later brought completely under control, necessitated this delay.

[7] Signs of external disease frequently appeared after prolonged exposure to extreme low temperatures, especially in specimens which had been conditioned to the higher acclimatization temperatures. Since it was desirable to avoid disease as a complicating lethal factor, and to deal only with the direct lethal effects of cold, determinations of lower limits of tolerance for periods longer than three days were not attempted with such specimens.

just able to withstand) for any given period of exposure is small. The difference between temperatures at which 100 per cent and 0 per cent survival was obtained averaged slightly over 2°, and in no case exceeded 3°. In contrast with this, marked differences of tolerance were shown by specimens conditioned to different temperatures. The lower median tolerance limits for the various fixed periods of exposure adopted (one to 72 hours) of fishes conditioned to 28° and to 20° differed, on the average, by 4.3°, and those of fishes conditioned to 20° and 12° by 4.0°. The corresponding average difference between the upper median tolerance limits of 20°- and 12°-conditioned specimens was 3.2°. There can be no doubt that these differences are significant. Only the heat-tolerance of specimens conditioned to 20° and to 28° did not differ appreciably.

The time-temperature curves of cold-tolerance (Figure 1) are dissimilar (not parallel). After 24 hours the lower median tolerance limits of 28°-conditioned specimens remained constant at 13°. This temperature may apparently be regarded as the "ultimate" or "true" lower median tolerance limit for these specimens, that is, the least extreme low temperature at which 50 per cent of the specimens are killed by cold, regardless of the duration of exposure. The ultimate limits of tolerance for specimens conditioned to 20° and to 12° cannot be stated, but can be judged by extrapolation to be slightly above 8° for the former, and somewhat above 5° for the latter.

It is noteworthy that, whereas for brief periods of exposure (one or three hours) the differences between the lower tolerance limits of fishes conditioned to 20° and 28° was considerably smaller than the difference between those of specimens conditioned to 20° and 12°, the reverse is true for longer periods of exposure (12 hours or more).[8] Short-time experiments obviously do not yield an entirely satisfactory measure of the changes of relative tolerance produced by acclimatization.

The time-temperature curves of heat-tolerance (Figure 2) tend to become parallel with the time axis sooner than the cold-tolerance curves. The ultimate or true upper median tolerance limits were apparently 31.4° for both the 28°-conditioned and 20°-conditioned specimens and 28.7° for the 12°-conditioned specimens. The absence of any difference between the 72-hour upper limits of tolerance of specimens conditioned to 28° and 20°, in contrast with a difference of

[8] The ratio (R) of the former difference to the latter $\left(R = \frac{T_m 28° - T_m 20°}{T_m 20° - T_m 12°}\right)$ increases with increasing time of exposure from 0.56 for one hour to 1.47 for 72 hours of exposure. When plotted against the logarithm of the duration of exposure to the test temperatures in hours ($\log t$), the values of R tend to fall roughly in a straight line ($R = 0.56 + 0.49 \log t$).

TABLE I

Per cent survival of Girella at various low temperatures and the estimated lower median tolerance limits (T_m) in relation to temperature of previous acclimatization and to time (duration) of exposure to the test temperatures

Date 1940	Acclim. temp. (° C.)	Test temp. (° C.)	No. of fish	Mean length (cm.)	Per cent surviving after							
					1 hr.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	120 hrs.
5/8	28°	13°	10	7.0	100%	100%	100%	100%	90%	50%	50%	
5/13	"	12°	11	7.1	100	100	91	45	0	0	0	
5/11	"	11°	10	7.1	100	100	90	30	0	0	0	
5/14	"	10°	10	7.2	100	100	0	0	0	0	0	
5/15	"	9°	10	7.2	100	90	0	0	0	0	0	
5/16	"	8°	10	7.2	80	10	0	0	0	0	0	
5/17	"	7°	10	7.0	20	0	0	0	0	0	0	
5/18	"	6°	8	7.1	0	0	0	0	0	0	0	
				T_m:	7.5°	8.5°	10.6°	12.1°	12.6°	13.0°	13.0°	
5/3	20°	8°	14	7.3	100%	100%	100%	100%	93%	64%	50%	
5/1	"	7°	12	7.2	100	100	100	58	17	0	0	
5/6	"	6°	14	7.3	100	93	36	0	0	0	0	
5/7	"	5.5°	12	7.2	75	42	0	0	0	0	0	
5/7	"	5°	14	7.3	0	0	0	0	0	0	0	
				T_m:	5.3°	5.6°	6.2°	6.9°	7.4°	7.8°	8.0°	
5/28	12°	5°	10	7.1	100%	100%	100%	100%	100%	100%	90%	50%
5/21	"	4°	11	7.1	100	100	100	100	100	45	0	0
5/25	"	3°	11	7.1	100	100	100	100	55	0	0	0
6/3	"	2°	10	7.2	90	70	60	10	0	0	0	0
6/4	"	1°	10	7.1	20	0	0	0	0	0	0	0
				T_m:	1.4°	1.7°	1.8°	2.4°	2.9°	4.1°	4.6°	5.0°

TABLE II

Per cent survival of Girella at various high temperatures and the estimated upper median tolerance limits (T_m) in relation to temperature of previous acclimatization and to time (duration) of exposure to the test temperatures.

Date 1940	Acclim. temp. (° C.)	Test temp. (° C.)	No. of fish	Mean length (cm.)	Per cent surviving after								
					1 hr.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	120 hrs.	Other periods
6/19	28°	33°	8	7.6	100%	100%	88%	0%	0%	0%	0%	0%	
6/21	"	32°	9	7.7	100	100	100	100	44	0	0	0	
6/24	"	31°	9	7.7	100	100	100	100	100	100	89	89	168 hrs.: 89%
			T_m:					32.5°	31.9°	31.5°	31.4°	31.4°	31.4° (168 hr.)
6/18	20°	35°	10	7.8	0%	0%	0%	0%	0%	0%	0%	0%	0.5 hr.: 50%
6/18	"	34°	12	7.9	100	0	0	0	0	0	0	0	
6/17	"	33°	12	7.8	100	100	25	0	0	0	0	0	
6/15	"	32°	12	7.7	100	100	100	75	0	0	0	0	
6/8	"	31°	12	7.8	100	100	100	100	100	83	83	83	
			T_m:		34.5°	33.5°	32.7°	32.3°	31.5°	31.4°	31.4°	31.4°	35.0° (0.5 hr.)
6/29	12°	31°	8	8.0	25%	0%	0%	0%	0%	0%	0%	0%	
6/28	"	30°	8	8.0	100	0	0	0	0	0	0	0	2 hrs.: 50%
6/22	"	29°	12	7.9	100	83	50	50	33	33	33	33	
6/19	"	28°	10	7.9	100	100	100	90	90	90	90	90	
6/14	"	27°	10	7.8	100	100	100	100	100	100	100	100	
			T_m:		30.7°	29.4°	29.0°	29.0°	28.7°	28.7°	28.7°	28.7°	30.0° (2 hr.)

5° between the corresponding lower limits, is of interest. A change of cold-tolerance produced by acclimatization is not necessarily accompanied by a corresponding change of heat-tolerance.

The lower the temperature of acclimatization, the earlier does the time-temperature curve of heat-tolerance tend to become parallel with

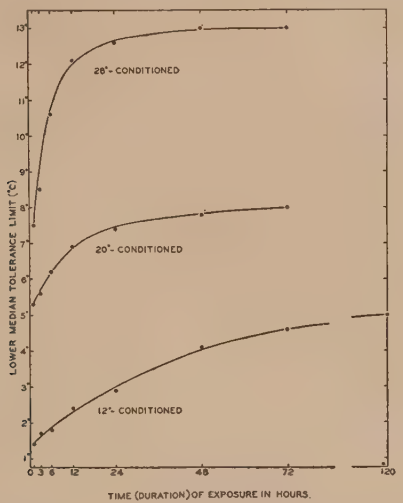
LOWER MEDIAN TOLERANCE LIMIT (°C)
28°- CONDITIONED
20°- CONDITIONED
12°- CONDITIONED
0 3 6 12 24 48 72 120
TIME (DURATION) OF EXPOSURE IN HOURS.

FIG. 1. Time-temperature curves of cold-tolerance: Lower median tolerance limits in relation to the time (duration) of exposure to the test temperatures. The specimens were previously acclimatized to three different temperatures (12°, 20° and 28°), indicated on each curve. Data from Table I.

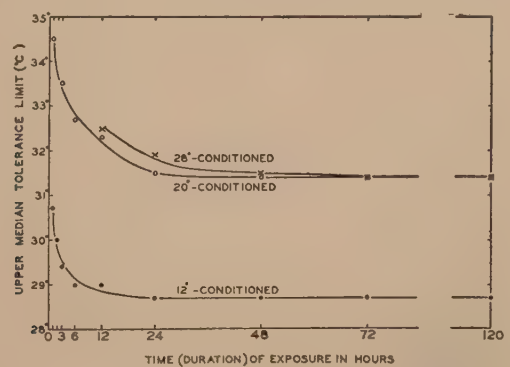

FIG. 2. Time-temperature curves of heat-tolerance: Upper median tolerance limits in relation to the time (duration) of exposure to the test temperatures. The specimens were previously acclimatized to three different temperatures (12°, 20° and 28°), indicated on each curve. Data from Table II.

the time axis. This is the reverse of the relationship between the corresponding curves of cold-tolerance (Figure 1). At slowly lethal temperatures the fishes probably undergo some acclimatization, the extent of which must vary with the magnitude of the change involved in the transfer to the lethal temperature. The differences noted above between the curves obtained with fishes acclimatized to different temperatures can be explained, at least in part, on the basis of this assumption.

The Rate of Acclimatization

Data already available relative to the rate of change of heat-tolerance of some marine fishes with acclimatization (Loeb and Wasteneys, 1912; Sumner and Doudoroff, 1938) indicate that increased heat-resistance is acquired rapidly after transfer to higher temperatures. While this change is reversible, heat resistance is apparently lost after transfer to lower temperatures much more slowly than it is gained. No detailed data were available on the corresponding rates of change of cold-tolerance, although a few observations bearing on this question have been made by Wells (1935) and by Sumner and Doudoroff (1938). The latter problem was now investigated in greater detail.

Limitations of material, time and facilities necessitated the adoption of some single test-period of exposure, for which tolerance limits were to be determined after varying periods of acclimatization to new temperatures. Within certain limits, the longer the period of exposure the more nearly should the results indicate the rate of change of the ultimate or true median tolerance limit. On the other hand, certain practical considerations made the use of a period longer than 24 hours undesirable, and this period was adopted.

Each test lot consisted of ten specimens, carefully selected with regard to size. The specimens varied in length between 5.6 cm. and 7.5 cm., and the mean length in each lot was 6.3 to 6.4 cm. Determinations of heat-tolerance were made in running water, and those of cold-tolerance in standing (aerated) water. The acclimatization temperatures used were 26°, 14° and, in a few experiments, 20°. The specimens were collected in the fall of 1940 and held at current sea-water temperatures. Some were transferred to 26° on October 7, some to 14° on November 16, and some to 20° on December 3.[9] After having been held at these respective (first) acclimatization temperatures for at least 42 days, lots of these specimens were transferred at convenient intervals of time to new (second) acclimatization temperatures. They were held in wire-screen cages immersed in the constant-temperature

[9] The sea-water temperatures on these dates were 19.7°, 17.3° and 16.5°, respectively.

TABLE III

*Per cent survival after 24 hours at various test temperatures and the estimated 24-hour median tolerance limits (T_m) of Girella, which had been acclimatized to various initial (first) temperatures and subsequently subjected for varying periods to other (second) acclimatization temperatures. The percentages are based on 10 specimens, except those marked *, which are based on 20 specimens. Where no second temperature is indicated, the fishes were transferred to the test temperature directly from the first acclimatization temperature. Tolerance limits marked (u) are upper, while the rest are lower median tolerance limits.*

Date of test	First acclim. temp.	Days at first temp.	Second acclim. temp.	Days at second temp.	Test temp.	Per cent surviving 24 hours	T_m (24-hour)
11/18/40	26°	42	—	0	11°	90	10.2°
11/20/40	"	44	—	0	10°	40	
11/25/40	"	49	14°	0.42	10°	80	9.3°
11/28/40	"	52	"	0.42	9°	40	
11/21/40	"	44	"	1	9°	100	8.4°
11/23/40	"	46	"	1	8°	20	
11/27/40	"	48	"	3	8°	90	7.2°
11/24/40	"	44	"	3	7°	40	
12/ 2/40	"	50	"	6	7°	100	6.4°
11/30/40	"	48	"	6	6°	20	
12/10/40	"	52	"	12	6°	90	5.5°
12/ 8/40	"	50	"	12	5°	10	
12/16/40	"	50	"	20	5°	80	4.4°
12/18/40	"	52	"	20	4°	30	
12/20/40	"	42	"	32	5°	90	4.3°
12/18/40	"	42	"	30	4°	30	
1/ 7/41	"	42	"	50	5°	100	4.2°
1/ 5/41	"	42	"	48	4°	40*	
12/11/40	"	65	—	0	11°	100	10.2°
12/ 9/40	"	63	—	0	10°	40	
12/13/40	"	65	20°	2	9°	90	8.2°
12/14/40	"	66	20°	2	8°	40	
12/31/40	14°	45	—	0	4°	60	3.8°
1/ 2/41	"	47	—	0	3°	0	
1/13/41	"	58	26°	0.15	5°	70	4.7°
1/14/41	"	59	"	0.15	4°	0	
1/10/41	"	55	"	0.42	6°	70	5.7°
1/12/41	"	57	"	0.42	5°	10	
1/ 7/41	"	51	"	1	7°	80	6.6°
1/ 4/41	"	48	"	1	6°	0	
1/ 8/41	"	51	"	2	8°	100	7.3°
1/ 8/41	"	51	"	2	7°	30	
1/ 9/41	"	51	"	3	8°	60	7.8°
1/ 7/41	"	49	"	3	7°	0	
1/12/41	"	51	"	6	9°	90	8.5°
1/14/41	"	53	"	6	8°	10	
1/20/41	"	53	"	12	10°	90	9.4°
1/18/41	"	51	"	12	9°	20	
1/28/41	"	53	"	20	10°	55*	9.9°
1/29/41	"	54	"	20	9°	0	

TABLE III—*Continued*

Date of test	First acclim. temp.	Days at first temp.	Second acclim. temp.	Days at second temp.	Test temp.	Per cent surviving 24 hours	T_m (24-hour)
1/31/41	14°	45	26°	31	10°	65*	9.8°
2/ 1/41	"	45	"	32	9°	0	
2/11/41	"	45	"	42	10°	50	10.0°
1/18/41	"	63	—	0	5°	100	4.2°
1/16/41	"	61	—	0	4°	40	
2/ 4/41	"	80	20°	0.42	5°	80	4.6°
2/ 6/41	"	82	"	0.42	4°	0	
1/20/41	"	63	"	2	6°	100	5.5°
1/22/41	"	65	"	2	5°	0	
2/ 1/41	"	69	"	8	7°	100	6.3°
1/31/41	"	68	"	8	6°	30	
1/16/41	20°	44	—	0	7°	65*	6.8°
1/20/41	"	48	—	0	6°	0	
2/ 1/41	"	60	14°	0.42	7°	100	6.2°
1/30/41	"	58	"	0.42	6°	40	
1/20/41	"	46	"	2	6°	100	5.4°
1/23/41	"	49	"	2	5°	10	
2/ 4/41	"	55	"	8	5°	100	4.5°
2/ 6/41	"	57	"	8	4°	0	
1/25/41	"	51	26°	2	9°	100	8.5°
1/27/41	"	53	26°	2	8°	0	
2/ 2/41	"	61	—	0	7°	50	7.0°
11/20/40	26°	44	—	0	31°	100	31.8° (*u*)
11/18/40	"	42	—	0	32°	40	
11/22/40	"	45	14°	1	32°	80	32.4° (*u*)
11/26/40	"	49	"	1	33°	0	
11/24/40	"	45	"	3	32°	80	32.4° (*u*)
11/27/40	"	48	"	3	33°	0	
12/23/40	"	42	"	35	30°	90	30.8° (*u*)
12/21/40	"	42	"	33	31°	40	
12/ 1/40	"	55	—	0	32°	50	32.0° (*u*)
1/ 1/41	14°	46	—	0	30°	70	30.5° (*u*)
1/ 6/41	"	51	—	0	31°	30	
1/ 8/41	"	52	26°	1	31°	100	31.7° (*u*)
1/10/41	"	54	"	1	32°	30	
1 15/41	"	57	"	3	31°	100	31.8° (*u*)
1/13/41	"	55	"	3	32°	40	
2/ 4/41	"	45	"	35	31°	100	31.7° (*u*)
2/ 2/41	"	45	"	33	32°	30	

tanks. Determinations of upper and lower 24-hour median tolerance limits were made with specimens taken directly from the first acclimatization temperatures, and with specimens which had been held for varying periods at the second temperatures. The experimental data and the estimated 24-hour median tolerance limits are presented in Table III. The tolerance limits are plotted against the duration of acclimatization to the second temperatures in Figures 3 and 4.

The difference between the upper limits of tolerance of specimens conditioned to 14° and to 26° was small (1.4°). With respect to the rate of change of heat-tolerance, the results obtained with Girella (Figure 3) are essentially in agreement with those which had been obtained with other species by other methods in earlier investigations. The 14°-conditioned fishes gained heat-tolerance rapidly after transfer to 26°. Acclimatization was apparently complete after about one day, the upper tolerance limit remaining constant thereafter.[10] On the other hand, the 26°-conditioned fishes showed no such rapid loss of heat-tolerance at 14°. Indeed, after one day they appeared to be somewhat more resistant than before, and this apparently increased resistance persisted for three days.[11] After 34 days at 14°, however, their resistance was considerably lower and did not differ significantly from that of fishes initially acclimatized to this temperature.

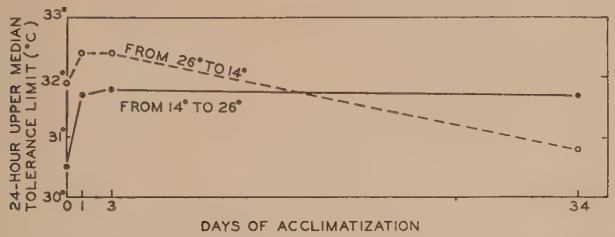

FIG. 3. Rate of change of heat-tolerance with acclimatization: The 24-hour upper median tolerance limits of Girella, initially acclimatized to 14° (solid circles) and to 26° (empty circles), in relation to the duration of acclimatization to new temperatures. The initial (first) and the new (second) acclimatization temperatures are indicated on each curve. Days of acclimatization (abscissas) refer to time at the second (new) temperatures only. The initial tolerance limit (0 days) of 26°-conditioned specimens is the mean of two determinations. Data from Table III.

The data on the rate of change of cold-tolerance are more detailed. The smooth curves which it was possible to fit to the experimental points (Figure 4) will be referred to as "acclimatization curves" of cold-tolerance. Although the curves are not identical in form, no very striking difference is to be noted between the rates of change of cold-tolerance following a corresponding rise and fall of temperature, such as was observed in the case of heat-tolerance. The tolerance limits of fishes transferred from 14° to 26° for acclimatization periods of 0.42 to 20 days may be shown to fall very nearly in a straight line when plotted

[10] Later experiments, which need not be presented in detail, indicated, however, that in two hours complete or even pronounced acclimatization did not occur.

[11] An indication of a similar slight gain of resistance was observed by Sumner and Doudoroff (1938) in Gillichthys. Its significance is too uncertain to warrant discussion.

against the logarithm of the duration of acclimatization. After 20 days at 26° complete acclimatization (i.e., a constant 24-hour lower median tolerance limit) was apparently achieved. The converse acclimatization curve of fishes transferred from 26° to 14° does not show the above logarithmic relationship. Acclimatization was somewhat slower during the first day, but, the curve being somewhat flatter, complete acclimatization again appears to be achieved in 20 days.

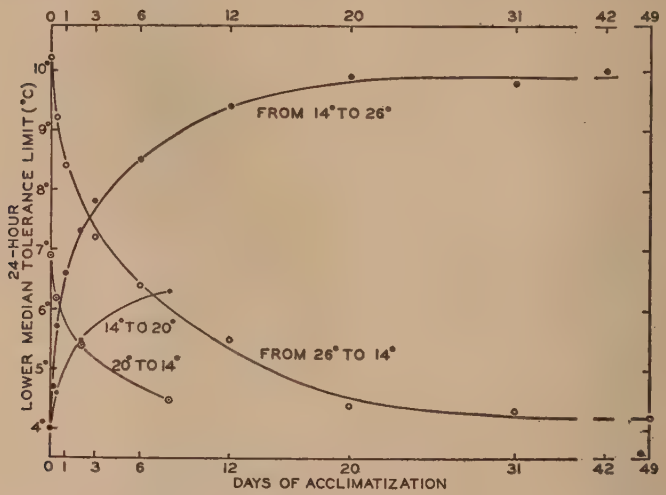

FIG. 4. Rate of change of cold-tolerance with acclimatization (acclimatization curves): The 24-hour lower median tolerance limits of Girella, initially acclimatized to 14° (solid circles), 20° (dotted circles) and 26° (empty circles) in relation to the duration of acclimatization to new temperatures. The initial (first) and the new (second) acclimatization temperatures are indicated on each curve. Points obtained with fishes transferred from 14° to 26° and to 20° are indicated by large and by small solid circles, respectively. Days of acclimatization (abscissas) refer to time at the second (new) temperatures only. The initial tolerance limit (0 days of acclimatization) is in each case a mean of two determinations. Data from Table III. The single determinations with fishes transferred for two days from 20° to 26° and vice versa are not plotted.

To what extent are the above acclimatization curves applicable to and descriptive of changes of cold-tolerance which result from smaller changes of temperature, such as may occur in nature? Although the available data for smaller changes (i.e., 14° to 20° and vice versa, etc.) are not complete, since complete acclimatization has not in any case been achieved, a satisfactory comparison can be made with corresponding portions of the curves obtained with larger temperature changes. The relationships can be illustrated by computing the fraction (per-

centage) of complete acclimatization achieved in each case after given periods (0.4, 2 and 8 days) of acclimatization to the second temperatures. The 24-hour lower median tolerance limits for fishes initially acclimatized to 26°, 20° and 14° and transferred directly to the test temperatures were 10.2°, 6.9° and 4.0° respectively. These values are means of the results of two experiments with each. They may, presumably, be taken to represent the tolerance limits after complete acclimatization. The percentages given in Table IV were calculated

TABLE IV

*Extent of acclimatization, expressed as per cent of total change of cold-tolerance, achieved in varying periods of acclimatization to new (second) temperatures after various changes of acclimatization temperature. (Values marked * were obtained by interpolation.)*

Acclim. temperatures		Per cent acclimatization achieved in:		
Initial (first)	New (second)	10 hours	2 days	8 days
14°	20°	21%	52%	79%
20°	14°	24%	52%	83%
14°	26°	27%	53%	79%*
26°	14°	16%	40%*	69%*
20°	26°		48%	
26°	20°		61%	

on this basis. The corresponding values in this table, that is, values for the same period of acclimatization, show no very striking differences and most show close agreement. In other words, the fraction of the total change of cold-tolerance (complete acclimatization) which occurs in any given time does not vary greatly with the direction or the magnitude of the total change. The somewhat low values obtained consistently with fishes transferred from 26° to 14° may be due to a certain amount of cold-injury, resulting from the sudden cooling to a temperature only slightly above their limit of tolerance. Such injury, while insufficient to cause visible distress, may account for the observed delay in the increase of resistance to further cooling. Gradual recovery, with adjustment to the new temperature, would account for the fact that complete acclimatization was not appreciably delayed, having been achieved in about 20 days, as in the case of the reverse change. Nevertheless, it appears that, at least for any moderate temperature change within the normal temperature range, the course of the acclimatization process (change of cold tolerance) can be described with reasonable accuracy as follows: about 50 per cent of the acclimatization

occurs in two days, and complete acclimatization is achieved in about 20 days, regardless of whether the temperature is raised or lowered.

This rate differs markedly from that reported by Mellanby (1939) for the change of chill-coma temperature in insects. This author usually obtained complete acclimatization in less than 20 hours, and at most in two or three days.

Discussion

It is uncertain to what extent temperatures may determine the distribution of species by virtue of their direct lethality. Non-lethal temperatures may be unsuitable for normal development, growth and activity, or for the activation of reproductive processes, or they may increase susceptibility to disease (e.g., see Staff, 1926). Furthermore, temperature may be a guiding stimulus in the movements of migratory fishes (Danois, 1924; Eggvin, 1937; Ward, 1921). Temperature selection by active fishes has also been demonstrated experimentally (Doudoroff, 1938), but the correspondence of these reactions to those under natural conditions is questionable. Since the distribution of many fishes may be confined to a narrower temperature range than that which is compatible with life, and they are not necessarily ever called upon to withstand temperatures near both their upper and lower limits of tolerance, it is interesting to compare the relative importance of lethal high and low temperatures as potential sources of danger to fishes in their natural habitats and, accordingly, as factors which may limit dispersal. While the opinion that fishes are more sensitive to warming than to cooling has sometimes been expressed or implied, it will be shown that injury by chilling deserves at least as much attention, as a limiting factor in the distribution of marine fishes, as heat-injury. This may not apply to fishes which are normally exposed in their habitat to temperatures at or near the freezing-point of their medium and are adapted to withstand them. Highest possible environmental temperatures are not so sharply defined, although in the open ocean 32° appears to be approximately the upper limit.

The destruction of marine fishes in large numbers in their natural habitat, apparently ascribable directly to unusual temperatures, has been reported repeatedly (Verrill, 1901; Storey and Gudger, 1936; and citations; Miller, 1940; Gunter, 1941). All such reports which have come to the writer's attention deal with destruction by extreme cold and not by heat. This circumstance already suggests a greater importance of lethal cold as a real hazard and as a factor limiting dispersal. Furthermore, of marine fishes which normally live at or near the surface, where temperatures are most variable, the more active and

sensitive ones may be able to escape abnormally high surface temperatures, but not low temperatures,[12] by means of a relatively short vertical migration.

In considering the experimental evidence the previous history of the experimental animals and the temperatures which are normal in their habitat must be taken into account. Maurel and Lagriffe (1899c) apparently failed to consider these factors, and hence their conclusions are of little value. Whether any given temperature should be regarded as more or less extreme than another depends upon its relationship to temperatures at which the fishes in question normally live. The mean surface temperature in the habitat from which the Girella were obtained is about 17°. The mid-value of the average yearly range (i.e., 13.3° to 22.0°), which for present purposes is also a significant point of reference, is 17.7°. Thus a temperature of 17.5° may be regarded as more or less typical of the habitat of young Girella in this locality. Specimens acclimatized to this temperature may be regarded as "normal," and temperatures equally removed from it as equally extreme or atypical. The limits of tolerance of normal Girella can be estimated by interpolation. Referring to Figure 5, the upper

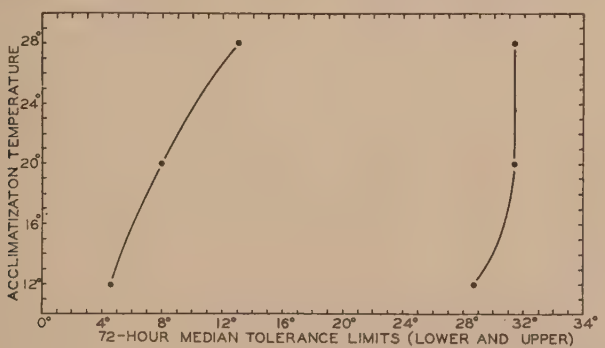

FIG 5. Relation between the temperature of previous acclimatization and the upper and lower 72-hour median tolerance limits. Curves fitted for approximate interpolation. Data from Tables I and II.

and lower median tolerance limits for 72 hours of exposure (the longest period for which complete data are available) corresponding to an acclimatization temperature of 17.5° are found to be about 31° and 6.8°, respectively. Thus the maximal rise of temperature tolerated for three days is by 2.8° greater than the maximal drop, and the lower limit of tolerance is, therefore, less extreme than the upper limit. With

[12] Unlike that of fresh water, the density of sea-water increases with decreasing temperature down to the freezing point.

longer periods of exposure the difference would be still greater. It seems safe to conclude that the fishes are no more resistant to cooling or to extreme low temperatures than they are to warming or to extreme high temperatures.

In nature fishes are not subject to such sudden changes of temperature as have just been considered. That changes of resistance to cold as well as to heat occur with gradual acclimatization has been amply demonstrated. In evaluating their significance, both their magnitude and their rate must be considered. The magnitude of the observed difference of tolerance resulting from a given difference between acclimatization temperatures was variable. The change of heat-tolerance was small in comparison with that of cold tolerance, especially because Girella, unlike other species which have been studied, showed no appreciable increase of heat-tolerance with acclimatization to temperatures above 20°. Possibly Girella could with gradual acclimatization survive cooling to as extreme a temperature as that to which it can be warmed. This, however, is uncertain, and the rise of temperature tolerated by specimens conditioned to the average yearly maximum (22°) is still greater than the drop tolerated by specimens acclimatized to the average yearly minimum (13.3°). This can be deduced from Figure 5. While few generalizations can be made it appears that the change of tolerance, both to heat and to cold, produced by a given change of acclimatization temperature is always considerably smaller than the latter change, and when the results obtained with long periods of exposure only are considered, it tends to decrease as the acclimatization temperature approaches the limit of tolerance.

The range of temperatures tolerated by "normal" Girella is greater than that to which fishes are exposed in the locality in which the specimens were taken. Therefore, the survival value of acclimatization is not obvious in this case. However, acclimatization may make possible the dispersal of a species over a wider range. Furthermore, much greater seasonal variations of surface temperature (i.e., as great as 20° to 28° C.) occur in some localities. Finally, the observed changes of resistance may be only more or less incidental manifestations of more fundamental adjustments of organisms to changes of environmental temperature, involving a general increase of vitality (resistance to disease, etc.) at non-lethal temperatures.

The physiological mechanism of the changes of resistance to heating and to chilling is not known. A change in the degree of saturation and, accordingly, of the melting and solidification points of protoplasmic lipoids has been suggested as a possible factor (Heilbrunn, 1937; Bělehrádek, 1935). The observations that in Girella a large change

of cold-tolerance could be obtained without a corresponding change of heat-tolerance, and that the rates of change of resistance to heat and to cold are quite different, indicate that these two changes are more or less independent of one another. Further investigation of the relative rates of physiological changes which occur with acclimatization (see also Sumner and Doudoroff, 1938) should prove to be of value in establishing which of these may be closely related and perhaps in revealing their mechanisms.

The ecological significance of the rate of acclimatization obviously depends upon the rate of fluctuation of environmental temperatures. In Girella even the relatively slow acclimatization to cold was complete after about 20 days. If this rate is typical, acclimatization should keep pace with seasonal temperature variations. However, large temperature fluctuations in marine environments resulting from the displacement of water masses, etc., may be quite rapid.[13] The gain of heat-tolerance of fishes apparently occurs very rapidly, while its loss on cooling is slow. The survival value of this relationship is obvious. Heat acclimatization will keep pace with a fairly rapid rise of temperature, and in an unstable (eurythermal) environment repeated brief sojourns at high temperatures may result in an increase of tolerance. On the other hand, acclimatization to cold in Girella was relatively slow, and the gain of cold-tolerance no more rapid than its loss. If this relationship is typical,[14] cold acclimatization should be relatively ineffective as a protective mechanism against occasional extreme temperatures in an environment in which fluctuations are rapid. These relationships can be advanced as further evidence that cold as a lethal agent may be more important as a factor limiting distribution than lethal heat.

The great sensitiveness of Girella and of other marine fishes to low temperatures or to cooling was further indicated by their reactions to temperature gradients (Doudoroff, 1938), in which the avoidance of low temperatures was much more pronounced than that of high temperatures. The temperatures most frequently selected by Girella which had been held at normal seasonal temperatures were 26° to 27°, i.e., much nearer their upper than their lower limits of tolerance. The fact that the reactions, just as heat-tolerance, were altered much more by acclimatization to very low temperatures than to high temperatures (Doudoroff, 1938, Figure 4) indicates a possible relationship between heat-resistance and selection in the gradient. However, the relative

[13] E.g., see Parr (1933), who reports changes of surface temperature in open water as great as 11° C. in two days.

[14] Some results of Sumner and Doudoroff (1938) indicate that it probably holds also for Gillichthys (see alternation experiments, loc. cit., pp. 421–422).

rates of acclimatization to higher and lower temperatures, as indicated by the reactions (Doudoroff, 1938, Figure 5), were more similar to the corresponding rates of change of cold-resistance than of heat-resistance.

Summary

The resistance of young *Girella nigricans* (Ayres) to low and to high temperatures and the changes of tolerance produced by acclimatization are examined in relation to temperatures in the natural habitat of the fishes.

The fishes were killed by moderately low temperatures well above 0° C., the lower limits of temperature tolerance being no more extreme, in comparison with normal environmental temperatures, than the corresponding upper limits.

The relationship between the time (duration) of exposure to the test temperatures and the highest or lowest temperatures tolerated by 50 per cent of the experimental animals (upper or lower "median tolerance limits") is representable by smooth time-temperature curves of tolerance.

Acclimatization to different temperatures has a pronounced influence upon subsequent resistance to cold (chilling), as well as to heat, and upon the form of the respective time-temperature curves of tolerance.

Experiments on the rate of acclimatization corroborate the conclusion, based on earlier observations, that heat-resistance in fishes is gained rapidly after a rise of temperature and lost slowly after cooling.

Acclimatization to cold (i.e., increase of resistance to chilling) is relatively slow, and cold-resistance is lost no more slowly than it is acquired. After any given rise or fall of environmental temperature within the normal range, about 50 per cent of the total resulting change of cold-tolerance occurs in two days, and complete acclimatization (i.e., constant cold-tolerance) is apparently achieved in about twenty days.

Injury by chilling is no less important as a possible limiting factor in the distribution of marine fishes than heat-injury.

LITERATURE CITED

Bělehrádek, J., 1935. Temperature and living matter. *Protoplasma-Monographien*, **8**: 277 pp. Berlin. Gebruder Borntraeger.

Binet, L. and G. Morin, 1934. Action de la chaleur sur les poissons. *Jour. de Physiol. et de Pathol. Générale*, **32**: 372–379.

Bodenheimer, F. S. and H. Z. Klein, 1930. Über die Temperaturabhängigkeiten von Insekten. II. Die Abhängigkeit der Aktivität bei der Ernteameise *Messor semirufus* E. André von Temperatur und anderen Factoren. *Zeitschr. f. vergl. Physiol.*, **11**: 345–385.

BORODIN, N. A., 1934. The anabiosis or phenomenon of resuscitation of fishes after being frozen. *Zool. Jahrb., Abt. f. allg. Zool. u. Physiol.*, **53**: 313–341.

BRITTON, S. W., 1924. The effects of extreme temperatures on fishes. *Am. Jour. Physiol.*, **67**: 411–421.

DANOIS, E. LE, 1924. Étude hydrologique de l'Atlantique-nord. *Ann. l'Inst. Océanogr.*, n.s., **1**: 1–52.

DOUDOROFF, P., 1938. Reactions of marine fishes to temperature gradients. *Biol. Bull.*, **75**: 494–509.

EGGVIN, J., 1937. Oceanographical conditions in North-Norway connected with the cod fisheries (Summary in English). *Fiskeridirektoratets Skrifter (Report Norw. Fish. and Mar. Investig.)*, **5**: 51–52.

FOX, H. M., 1939. The activity and metabolism of poikilothermal animals in different latitudes. *Proc. Zool. Soc.*, **109**: 141–156.

GUNTER, G., 1941. Death of fishes due to cold on the Texas coast, January 1940. *Ecology*, **22**: 203–208.

HATHAWAY, E. S., 1927. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. *Bull. Bureau of Fisheries*, **43**, II: 169–192.

HEILBRUNN, L. V., 1937. An outline of general physiology. Philadelphia. W. B. Saunders Co., 603 pp.

HÖRSTADIUS, S., 1925. Temperaturanpassung bei den Eiern von *Paracentrotus lividus* Lk. *Biologia Generalis*, **1**: 522–536.

HUNTSMAN, A. G., 1926. The comparative thanatology of marine animals. *Trans. Roy. Soc. Canada*, **20**, V: 187–208.

HUNTSMAN, A. G. AND M. I. SPARKS, 1924. Limiting factors for marine animals. 3. Relative resistance to high temperatures. *Contrib. Canadian Biol.*, n.s., **2**: 97–114.

JORDAN, D. S., B. W. EVERMANN AND H. W. CLARK, 1930. Check list of fishes and fish-like vertebrates of North and Middle America north of the northern boundary of Venezuela and Colombia. *Report of the U. S. Commissioner of Fisheries, 1928*, II, **1055**: 670 pp. Washington, Government Printing Office.

LOEB, J. AND H. WASTENEYS, 1912. On the adaptation of fish (Fundulus) to higher temperatures. *Jour. Exp. Zool.*, **12**: 543–557.

LUYET, B., 1938. Sur la survie des poissons plongés dans l'air liquide. *C. R. Soc. Biol.*, **127**: 788–790.

LUYET, B. AND P. M. GEHENIO, 1938. The lower limit of vital temperatures—a review. *Biodynamica*, **33**: 1–92.

MAUREL AND LAGRIFFE, 1899a. Determination et action des plus hautes températures compatibles avec la vie de certains poissons. *C. R. Soc. Biol.*, **51**: 797–800.

MAURELL AND LAGRIFFE, 1899b. Determination et action des plus basses températures compatibles avec la vie de certains poissons. *C. R. Soc. Biol.*, **51**: 875–878.

MAUREL AND LAGRIFFE, 1899c. Action comparée de la chaleur et du froid sur certains poissons. *C. R. Soc. Biol.*, **51**: 915–918.

MELLANBY, K., 1939. Low temperature and insect activity. *Proc. Roy. Soc. London*, B, **127**: 473–487.

MELLANBY, K., 1940. Temperature acclimatization in amphibia. *Jour. Physiol.*, **98**: 27–28 P (*Proc. Physiol. Soc.*).

MILLER, E. M., 1940. Mortality of fishes due to cold on the southeastern Florida coast, 1940. *Ecology*, **21**: 420–421.

OGLE, C. AND C. A. MILLS, 1933. Animal adaptation to environmental temperature conditions. *Am. Jour. Physiol.*, **103**: 606–612.

PARR, A. E., 1933. A geographic-ecological analysis of the seasonal changes in temperature conditions in shallow water along the Atlantic coast of the United States. *Bull. Bingham Oceanogr. Collection*, **4**, (3): 90 pp.

PAYNE, N. M., 1926a. The effect of environmental temperatures upon insect freezing points. *Ecology*, **7**: 99–106.

PAYNE, N. M., 1926b. Freezing and survival of insects at low temperatures. *Quart. Rev. Biol.*, **1**: 270–282.

REGNARD, P., 1895. Action des très basses températures sur les animaux aquatiques *C. R. Soc. Biol.*, **2**: 652–653.

SCHMIDT, P. J., G. P. PLATONOV AND S. A. PERSON, 1936. On the anabiosis of fishes in supercooled water. *C. R. Acad. Sci. U.R.S.S.*, **3**: 305–308.

STAFF, F., 1926. Maladie des narines de la carpe. Contribution sur la physiologie et pathologie du sommeil hivernal. *Internat. Rev. de Hydrobiol.*, **14**: 146–159, 312–321.

STOREY, M., 1937. The relation between normal range and mortality of fishes due to cold at Sanibel Island, Florida. *Ecology*, **18**: 10–26.

STOREY, M. AND E. W. GUDGER, 1936. Mortality of fishes due to cold at Sanibel Island, Florida, 1886–1936. *Ecology*, **7**: 640–648.

SUMNER, F. B. AND P. DOUDOROFF, 1938. Some experiments on temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, **74**: 403–429.

SUMNER, F. B. AND M. C. SARGENT, 1940. Some observations on the physiology of warm spring fishes. *Ecology*, **21**: 45–54.

U. S. Weather Bureau, 1938. Atlas of climatic charts of the oceans. *W. B. no.* **1247**: 130 pp.

VERRILL, A. E., 1901. A remarkable instance of the death of fishes at Bermuda in 1901. *Am. Jour. Sci.*, 4, **12**: 88.

WARD, H. B., 1921. Some of the factors controlling the migration and spawning of the Alaska red salmon. *Ecology*, **2**: 235–254.

WEIGMANN, R., 1929. Die Wirkung starker Abkühlung auf Amphibien und Reptilien. *Zeitschr. f. wissenschaft. Zool.*, **134**: 641–692.

WEIGMANN, R., 1930. Weitere Untersuchungen über die Kältebeständigkeit poikilothermer Wirbeltiere. *Zeitschr. f. wissenschaft. Zool.*, **136**: 195–209.

WEIGMANN, R., 1936. Zur Kältebeständigkeit poikilothermer Tiere, Untersuchung an Schnecken und Fischen. *Biol. Zentralblatt*, **56**: 301–322.

WELLS, N. A., 1935. Variations in the respiratory metabolism of the Pacific killifish *Fundulus parvipinnis* due to size, season, and continued constant temperature. *Physiol. Zool.*, **8**: 318–336.

WHITNEY, R. J., 1939. Thermal resistance of mayfly nymphs from ponds and streams. *Jour. Exper. Biol.*, **16**: 374–385.

Contribution No. 175

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RESEARCH WITHIN PHYSICAL OCEANOGRAPHY AND SUBMARINE GEOLOGY AT THE SCRIPPS INSTITUTION OF OCEANOGRAPHY DURING APRIL 1941 TO APRIL 1942

H. U. Sverdrup and Staff

(Contribution from the Scripps Institution of Oceanography, New Series, No. 175)

Again emphasis will be placed on the activities in the fields of physical and chemical oceanography and submarine geology, although researches within the biological sciences play a prominent part at the Scripps Institution of Oceanography. The compilation and discussion of field-data and the studies in the laboratory were greatly facilitated until the end of December 1941 by assistance obtained from the Work Projects Administration Project No. 65-1-07-2317.

Within physical oceanography and submarine geology the field- and research-work have been greatly influenced by the war. Since July 1, 1941, the E. W. Scripps has been at the disposal of a University of California Defense Research Project, and several staff-members have been attached to that project.

The six cruises which were planned for the spring of 1941 in cooperation with the United States Fish and Wildlife Service were completed, however, and showed the same general development of the currents as described in the report of 1939, but with different details. The data from these cruises have been partly worked up under the supervision of Messrs. C. E. LA FOND and R. B. TIBBY.

W. C. JACOBS completed an examination of the evaporation from the oceans and the exchange of heat between the ocean and the atmosphere, making extensive use of the data contained in the Atlas of Climatic Charts of the Oceans, published by the United States Weather Bureau in 1938.

G. F. McEWEN has continued the special study of the Colorado River runoff, referred to in the report of 1940-41. In order to advance this work a conference was held at the Scripps Institution on September 5 and 6, 1941, which was attended by representatives of the Bureau of Power and Light of the City of Los Angeles, Professor A. E. DOUGLASS of the Laboratory of Tree Ring Research, University of Arizona, and by representatives of the University of California.

McEWEN has also made progress in dealing with the problem of the horizontal dispersion of material, for example, plankton or fish eggs from an initial source, circular in form and of any given radius. This problem arose in fishery research. Although it has not proved possible exactly to solve this problem--a generalization of the point-source problem whose solution is well known--three different general solutions have been worked out which furnish results approximating closely to the true result.

A generalization has been worked out of the problem of sedimentation from a line-surface source at shore, assuming a constant settling rate and horizontal and vertical diffusion having different values of the diffusion-coefficient. Two general solutions have been worked out for the general case of a continuous-line source not restricted as before to the steady state. This is of special interest to geologists. The solutions found are close approximations.

Conferences have been carried on with Dr. SETTE of the United States Fish and Wildlife Service on statistical aspects of fishery problems, and with biologists at the Scripps Institution on the application of statistical theory to their work.

The statistical theory of the precipitation-ratio used in Dr. GORCZYNSKI'S investigations of climate has been extended and generalized to cover other statistical problems of the maximum and minimum values of a variable having different frequency-distributions.

The climatological investigation by GORCZYNSKI and McEWEN, described at some length in the report of 1940-41, has continued. The main effort in this field has been devoted to preparing the manuscript of the book, "California's climate compared with those of other sunny lands."

Daily observations of temperature, salinity, and weather have been obtained from six shore-stations, including the station at the end of the Scripps Institution Pier. Supplementary observations from United States Lighthouse tenders and tuna clipper-ships have been collected. At the Scripps Institution continuous barograph and pyrheliometer-records, as well as daily observations of humidity and precipitation, have been made.

Because, during most of the time since July, 1941, clerical help was greatly reduced and wa mainly occupied in preparing the manuscript for the book on climate, special attention was give to certain mathematical problems arising in the fields of climatology and oceanography in order to provide the necessary foundation for numerical applications to be carried out later. Encoura ing progress has been made in this undertaking.

Under the direction of E. G. MOBERG two investigations of importance in the physical chen istry of sea water were initiated. One of these, carried out by JAMES C. HINDMAN, deals with much-investigated but still unsolved problem of the solubility of calcium carbonate in sea water and is a corollary of the work of JOHN LYMAN on the buffer-mechanism, particularly on the dis sociation constants of carbonic and boric acids, in sea water and which was reported upon in 19 Mr. HINDMAN obtains the data for the calculation of the solubility-product constants of the carb ates of calcium and other alkaline earths by titration of their chlorides or nitrates with sodium bonate by methods and under conditions capable of yielding results of a high degree of accuracy. By this procedure certain peculiarities in the behavior of calcium carbonate have been observed These will probably explain the divergent views regarding the relative concentration of calcium carbonate in sea water that have been published.

The other investigation was undertaken with a view of finding a method for determining the chlorinity of sea water more accurately than is possible with the standard KNUDSEN method. Such a method was especially needed for evaluating reference (standard) sea-water samples wh are no longer obtainable from the usual source. A potentiometric titration-method was adopted, and to serve as a titration end-point indicator a vacuum-tube voltmeter of high sensitivity and wide range of application was designed and constructed by L. J. ANDERSON and J. C. HINDMAN. A paper describing the construction and uses of this voltmeter has been prepared.

A novel feature of this potentiometric titration-method is the introduction of the reversible silver-silver chloride electrode as the indicating electrode and an electrode of platinum immersed in KNO_3 and sealed in glass as the reference. Contact with the solution is made throug an asbestos filter. Using these electrodes together with the voltmeter above mentioned and a weight-technique with mechanical agitation, a precision in the determination of chloride of ± 0.0 per mille has been obtained. A comparison of Copenhagen standard sea-water with a primary sodium-chloride standard has been made. The results obtained show a difference of only 0.002 per mille between these standards. The results indicate that this method and standard sodium chloride have much to recommend them. The time involved in the titration is far less than in th VOLHARD procedure and the accuracy and precision are greater. Comparisons were also mad with the colorimetric MOHR (or KNUDSEN) method using similar technique. Excellent agreeme was obtained indicating that under proper conditions the MOHR titration is capable of high preci sion.

The work of the Sediments Division has been greatly reduced because R. R. REVELLE, in charge of the Division, was called to active duty in the Navy on July 10, 1941. Routine analyses were carried out by L. J. ANDERSON with the help of a Work Projects Administration assistant until the discontinuance of the project at the Institution in December. About 250 individual samples of marine sediments were analyzed for particle-size distribution and for water-content dur the nine-month period. The water-content analyses were made first on the basis of the weight-l on drying, and then on the basis of the chloride content of the sample. Analyses for chloride we made on all samples by means of a conductometric method developed in the Division. This met eliminates the necessity of leaching the chloride out of the mud before analysis because the pre ence of mud-particles does not interfere with the electrically-determined end-point of the silve nitrate titration. About 20 samples were analyzed for nitrogen and sulfur in connection with investigations undertaken by REVELLE and RITTENBERG on the sulfur cycle in marine sediment The procedure used for determining calcium carbonate in the sediments was improved and work was started on the development of another one based on a different principle.

Intermittently, during the entire year a conductometric method for determining sulfate in s water and marine mud has been improved. The main substance that interferes is calcium, whic coprecipitates with barium sulfate in the titration. Convenient ways of removing the calcium ar being investigated at present.

Other investigations by L. J. ANDERSON on potentiometric titration were described above when reporting on work in the division of chemistry.

F. P. SHEPARD has prepared a series of charts of the Gulf of California and adjacent terri tory, incorporating in them the 25,000 soundings of the E. W. Scripps Expedition of 1940 to the

Gulf and the 3,000 soundings of the Expedition of 1939. These charts have been contoured and are now ready for publication. The report on the submarine topography of the Gulf of California has also been written. This report contains a discussion of the numerous fault-scarps discovered on the floor of the Gulf, with suggestions of the history of earth-movements which these scarps indicate. Also the submarine canyons of the Gulf are considered at some length. The localization of these canyons in the more humid areas is considered and the relation between these canyons and the diastrophic history is discussed.

As far as possible, work has continued on the study of beach-erosion and long-shore drift of sand in La Jolla Cove. Currents were observed daily at 14 stations in the Bay during the past summer. Analyses of the wave-records obtained daily at the Institution Pier have been made with the cooperation of E. C. LA FOND and W. F. WOHNUS and show that the waves have a period which averages about 9.2 seconds. Storm-waves in general have shorter periods and the small wind-waves in summer also have short periods. Erosion of the beach and foreshore show direct relation to the character of the waves. Direct relations between quantity of mica on the beach and quantity of heavy black sands to the size of the waves were also established, the mica decreasing and the black sand increasing with increase in size of waves. Also, mica was found to occupy the troughs of ripples on the beach and black sand on the seaward slope of the ripple-marks. More evidence was obtained showing the importance of the location of rip-currents to beach-erosion during the stormy season.

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Contribution No. 176

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THE KELP RESOURCES OF SOUTHERN CALIFORNIA[1]

By J. F. WOHNUS
Scripps Institution of Oceanography

During the years 1916-1918 the large beds of *Macrocystis pyrifera* which occur off the Pacific coast supported an extensive industry. Commercial interest in kelp predated this period by a great many years but it remained for World War I to stimulate active interest in the utilization of products obtained from the domestic kelp beds. During this first period of the California kelp industry the basic products obtained from processing kelp were iodine, inorganic salts, and organic solvents. The most important of these products were high grade (95-98% pure) potassium chloride, acetone, ethyl acetate, and the amyl acetate substitutes, ethyl propionate and ethyl butyrate.

Two very different methods were devised to obtain these products. The first or so-called char method was used primarily to obtain potassium chloride, sodium chloride, iodine, and a high grade of decolorizing carbon. This was the simpler of the two methods and may be described best by a plant flow sheet diagram (Fig. 53) showing the principal steps involved. The second or fermentation process was developed by the Hercules Powder Company.

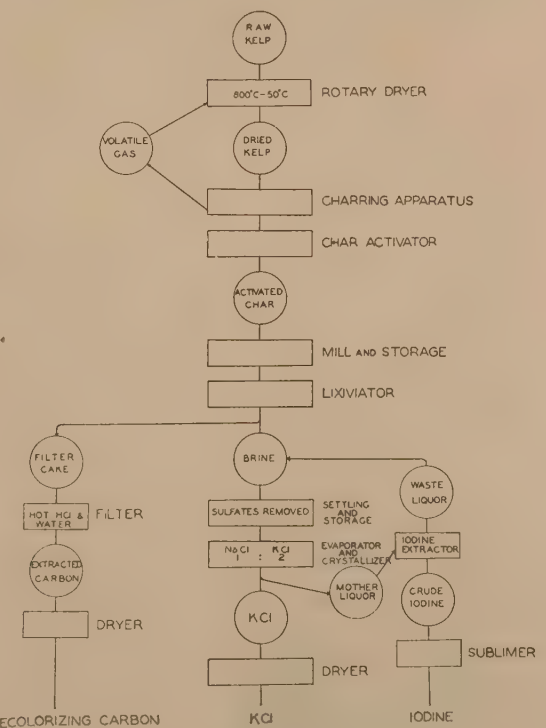

FIG. 53. Plant flow sheet diagram showing the main steps in the char method of processing kelp.

[1] This paper was presented at the symposium "Resources of the sea for wartime economy" at the meeting of the Oceanographic Society of the Pacific, held in Salt Lake City, Utah, June 17, 1942. (Contributions from the Scripps Institution of Oceanography, New Series, No. 176.)

This procedure involved different methods of handling the raw-products and necessitated a tremendous amount of research work on the part of this company. One of the outstanding features of the Hercules plant was its Tank Farm for the initial fermentation. This consisted of 150 tanks, each of a 50,000 gal. capacity. Aside from this feature the process again may be described by a flow sheet diagram (Fig. 54).

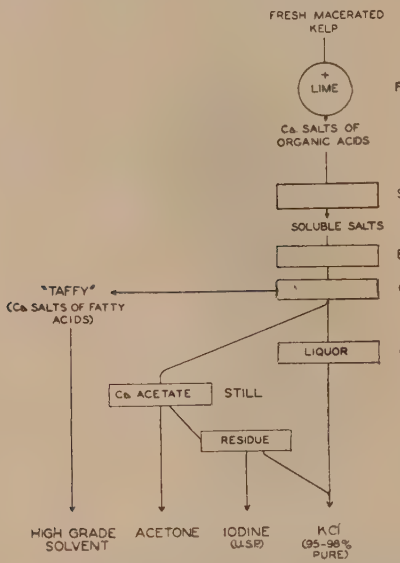

FIG. 54. Plant flow sheet diagram showing the principal steps in the fermentation process developed by the Hercules Powder Company.

It is of interest to point out that discussions of this first phase of the kelp industry emphasize that wartime economy was necessary to enable these industries to function and that the only claim to permanence which could be made for the kelp industry was the fact that the products were all of a high degree of purity. This is also demonstrated by the fact that nearly all of the companies stopped operations immediately following the war. In the Facts of Current Interest column of "California Fish and Game" for October, 1921, the following statement appears, "The kelp industry, which grew to importance during the war, is now a memory, all the plants having closed down."

In reporting this fact it would have been better in the light of later developments to state that the first phase of the kelp industry was over. Actual production had ceased but there were still a group of people actively interested in utilizing kelp as a natural resource.

In addition to the utilization of kelp as a source of chemical products, the dried and ground material was found to be a good chicken, fox, and cattle food supplement. This usage aided materially in maintaining and renewing interest in the kelp industry. Moreover, uses were being found for a group of products previously believed to be chemical oddities. These products, the alginates, are metallic salts of alginic acid and have proven to be of value in a number of diverse fields. New uses are being found for these products and this industry, although small in comparison with the fisheries, is certainly making valuable contributions to the war effort.

The processing of kelp to obtain alginates utilizes a modification of the wet process used during the early years of the kelp industry of

Scotland. Here again the use of a flow sheet (Fig. 55) enables one to examine and describe the process. In brief the process now employed utilizes the organic constituents of the kelp plant, and the products previously prepared are not utilized.

Since the alginates have such a diverse application a brief summary of some of their uses is of interest. These may be arbitrarily divided into three groups. The first includes those which are primarily for a peace time economy. The second group includes those which were in use prior to the outbreak of war but have direct application in the war effort. The third group consists of the applications developed since the beginning of war and are essential for war purposes.

In the first group the utilization of sodium alginate as a stabilizer for dairy products, ice creams and chocolate milk, would be considered most important. In addition the use of sodium alginate as an ingredient in cosmetics and dentifrices would be considered as would that of less pure and unbleached sodium alginate as a dye vehicle for cloth printing and as a binder for printers ink.

The second group includes the use of ammonium alginate in cream latex to increase the solid content and prevent the coagulation of rubber shipments; the addition of alginates to asphalt paints for steel plates and insulated wires to prevent the painted surfaces from adhering to each other; and the use of sodium alginate as an ingredient in boiler compounds to prevent or retard the development of boiler scale. In addition the use of alginates by baking companies in the preparation of jams and jellies is being increased under the existing lend-lease program.

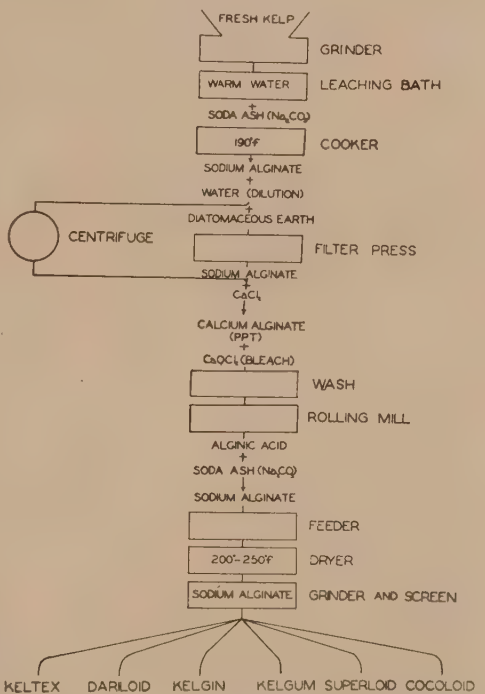

FIG. 55. Plant flow sheet diagram showing the principal steps in the modified wet process in use at the present time.

The third group consists of the utilization of alginates as stabilizers for camouflage paints and of copper alginate as a dressing for canvas and burlap to prevent mildew. Under the stress of war necessity new and more important uses are being sought and without doubt many more will be found which will have a direct or indirect application to the current war effort.

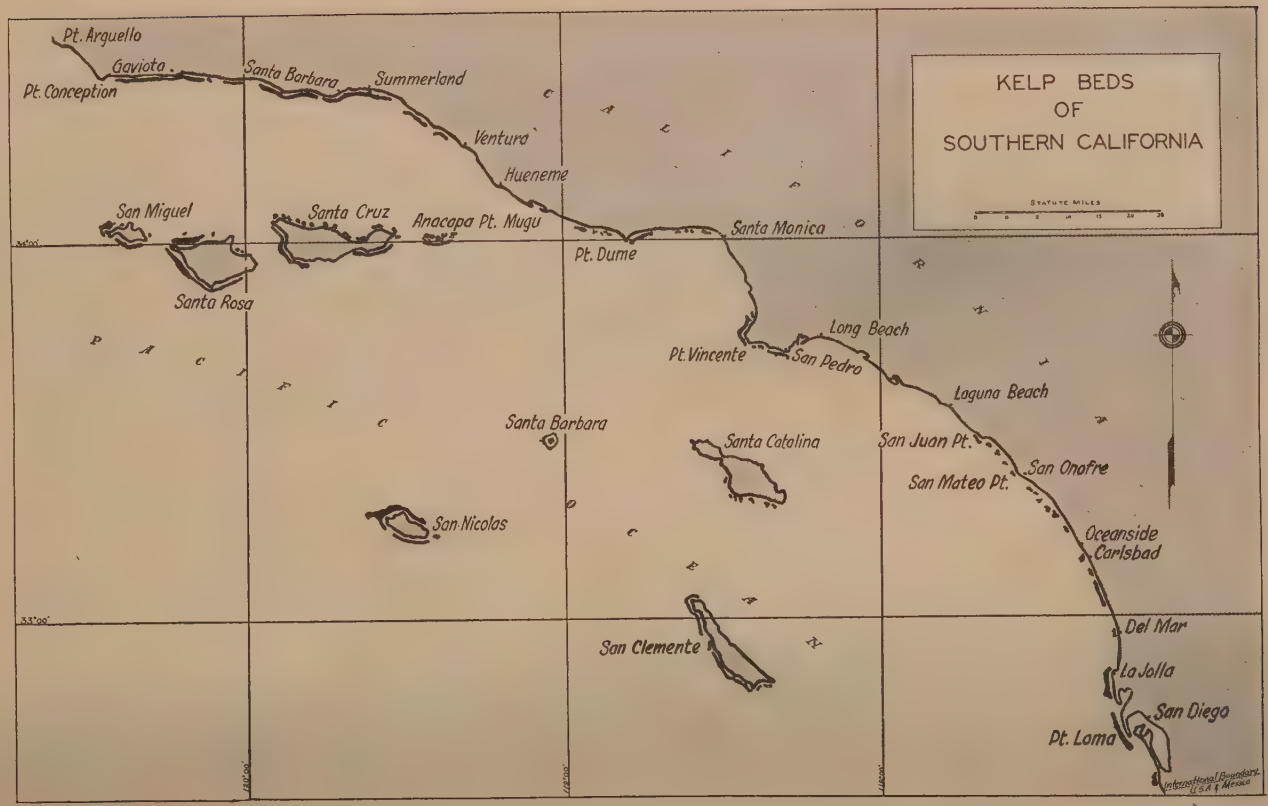

FIG. 56. Distribution of kelp in southern California. (Kelp beds shown in solid black.)

Mention should be made of another important use, namely, as a substitution product. With the curtailment of agar imports and the accompanying demand placed on local industries it has been found that alginates may be used for a number of purposes previously reserved for agar. Thus the dental profession has employed alginate-agar mixtures for denture impressions. It has also been found that these mixtures may be used for bacteriological gels. This "dilution" of the agar supply will doubtless have the effect of making the existing stock of this essential material go further.

In any industry dependent on natural resources it is natural to be concerned over the supply of that resource. During the past two years increasing concern has been felt concerning the kelp supply. Certain kelp beds had decreased alarmingly in size as well as density and during the past year a study has been made of this situation.

TABLE 1. CHANGES IN AREAS OF THE KELP BEDS IN THE LA JOLLA-SAN DIEGO REGION

Bed	Year	Area	Change over 1911		Change over 1934		Remarks
			Actual	Per cent	Actual	Per cent	
2	1911	1.4	----------	----------	----------	----------	Heavy
	1934	0.88	—0.52	—37%	----------	----------	Scattered
	1941	(Est.) 0.5	—0.9	—66%	—0.38	—43%	Very thin
3	1911	4.08	----------	----------	----------	----------	Heavy
	1934	2.77	—1.31	—32%	----------	----------	----------
	1941	2.14	—1.94	—47.5%	—0.63	—22.7%	Thin
4	1911	2.1	----------	----------	----------	----------	Heavy
	1934	2.6	+0.5	+23.8%	----------	----------	----------
	1941	2.5	+0.4	+19%	—0.1	—3.8%	Medium

A map (Fig. 56) of the southern California coastline will serve to show the distribution of the important harbors: Hueneme, San Pedro and San Diego. It can be seen at once that the kelp beds are found close to the shore of the mainland and of the offshore channel islands. In general, the beds in the southern California region are restricted to water of five to fifteen fathoms and their location with respect to distance from the shore line is governed in part by the occurrence of water of this depth. Actually the distance from any particular kelp bed to the adjacent shore is seldom greater than one mile. Much of the survey has been curtailed with the restrictions placed on offshore boat travel and a great deal of important information is lacking. However, the San Diego region (Fig. 57) has been carefully surveyed and compared with previous surveys and the results may be shown in table form (Table 1). The remaining areas supporting growth of kelp have been discussed with the captains of the kelp harvesters and fishermen, and, in general, it may be stated that there are definite signs of improvement in the condition of the kelp beds. In some areas they have not yet regained the extensive growth described in previous surveys, but, at the same time, there are a few regions in which kelp is now

growing where it had not been reported previously. With care and proper regulation it is believed that the supply of this natural resource can be maintained and the present yield increased.

The San Diego region, although it represents approximately 10 per cent of the entire kelp bed area, presents many important considerations. The increase in kelp as one proceeds north along the shore is of interest, especially if it is pointed out that Bed 4, off La Jolla, has yielded the most kelp in the past year, whereas Bed 2, off Pt. Loma, further south, has not been harvested.

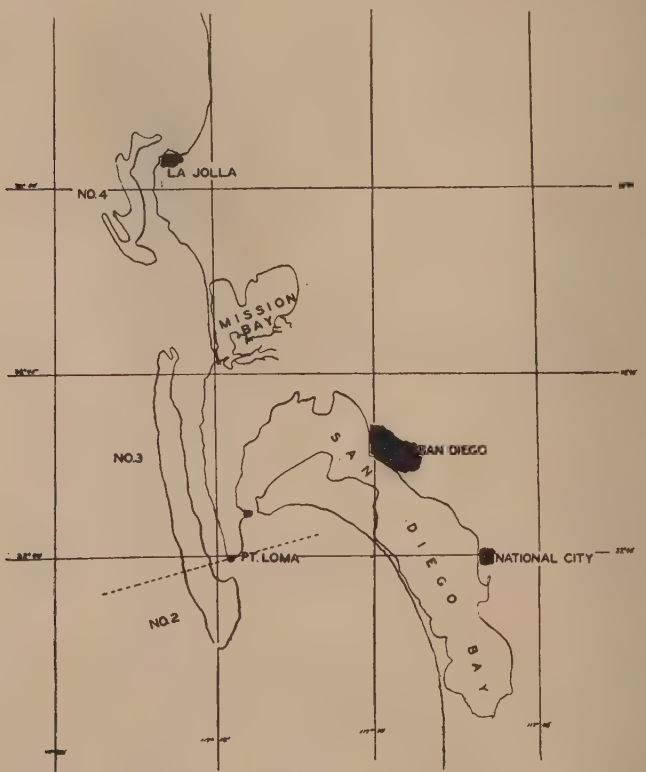

FIG. 57. Map of the La Jolla-San Diego region showing the location of the kelp beds in that area.

Preliminary examination of this region by divers has shown that bottom conditions are all satisfactory for the growth of kelp. It therefore remains to hypothesize why the area of kelp in this region has been reduced so much. The statistics of the changes in area show that this reduction has taken place over a rather long period of time and it may well be that the present condition represents the culmination of a serious alteration in environmental conditions.

Located as it is at the mouth of a bay (San Diego Bay), Bed 2 is subjected to a great many factors which are not present in the other kelp beds. Siltage (producing poor light conditions), contamination,

and lack of nutrients may have united to produce the reduction in the area of kelp in this region. It is of interest to point out that the kelp in this region has shown an increase during the past six months, which may reflect an improvement in some one of these basic conditions.

During this discussion the attempt has been made to demonstrate the contributions which can be made by the kelp industry to the war emergency. This may require diverting some of its products from civilian uses to those considered essential to the war effort. With the possibility of an improved supply of raw material, and with the work being done in seeking new applications for its products, certainly an optimistic view may be held for the California kelp industry.

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THE DEVELOPMENT OF THE SPOROPHYTE OF MACROCYSTIS PYRIFERA

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Contributions from the Scripps Institution of Oceanography
New Series, No. 177

During the course of a series of kelp investigations conducted through the past year it was possible to obtain a complete series of specimens which show graphically the development of the sporophyte of *Macrocystis pyrifera*. This giant kelp of the Pacific Coast has an extensive distribution as pointed out by Setchell and has also been the subject of many studies notably those of Setchell, Setchell and Gardner, Skottsberg, and Brandt, but none of these is accompanied by as complete a series of specimens as presented here. Therefore, although a great deal of the description of the development has been reported in these papers, it is believed that the instructional value to be obtained from presenting this series as a unit offsets the disadvantages of repetition.

The arrangement of a series of this nature is more or less arbitrary, but it is essential to select the individuals which show most clearly the sequence of events in the development of the mature plant. No single criterion such as size, state of development of the holdfast, or degree of splitting in the formation of the leaves is infallible, but in preparing the arrangement of this series the latter two were used. Size of the individual proved to be too variable to be a practical guide. It is natural to expect variations in size depending on fluctuations in the environment, the amount of crowding, as well as other growth stimulating or inhibiting factors operating. In any case, it is believed that the following series shows most clearly the development of the sporophyte of *Macrocystis pyrifera*.

Figures 1, 2, 3, and 4 are of specimens probably not more than one and one half to two months of age and depict the early development of a single bladed plant. In figure 5 there is the beginning of the formation of the first split in this blade which in figure 6 is seen to have elongated and partially divided the blade into two relatively symmetrical halves.

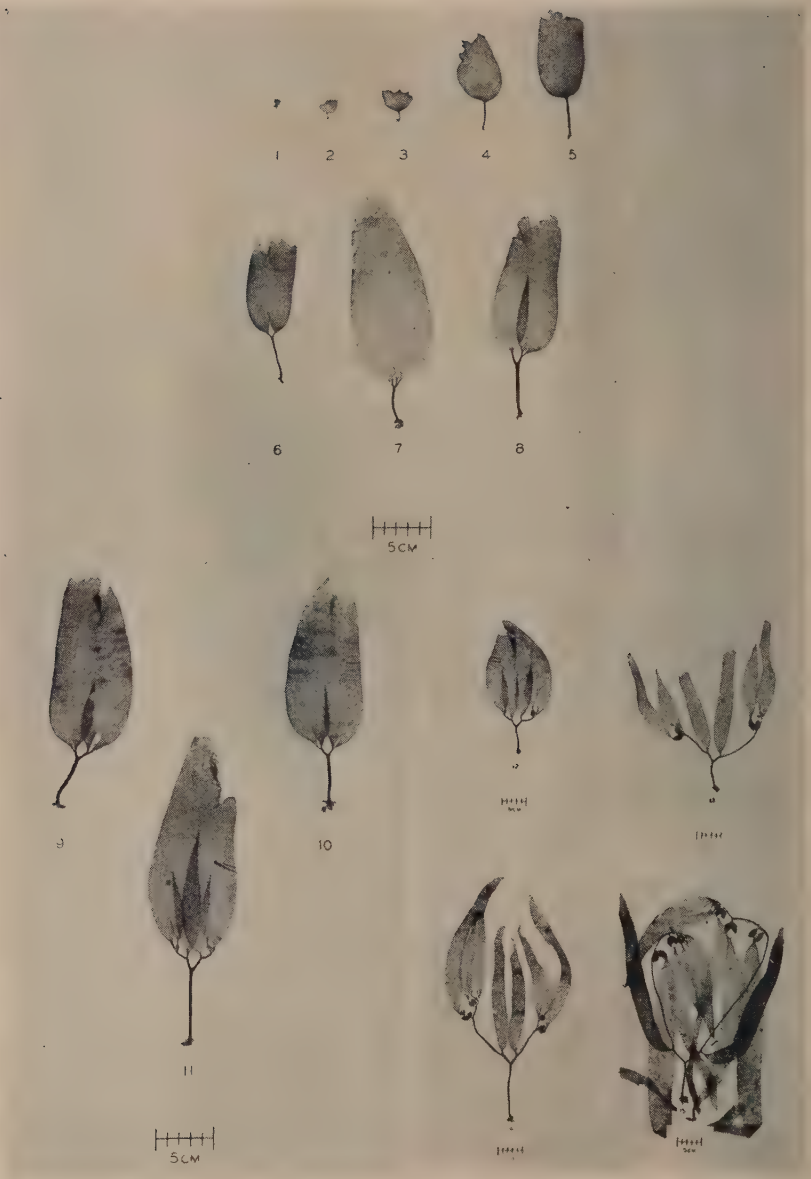
Another instance of the minor variability which occurs in the development is shown in figure 7 where actually the entire plant is larger than the subsequent stage but the development of the first split has not progressed as far as in figure 6, and, in addition, the development of the second split has already occurred in figure 7.

Figures 8 and 9, of plants approximately two and one-half to three months of age, show the progressive elongation of the primary split, the establishment of the second split, and the development of the primary stem. Up to this time there has been a marked symmetry in the appearance and development of the clefts destined to form the leaves. In figure 10, however, a minor asymmetry is noted in that the third division is apparent on the frond on the left side of the specimen for which there is no counterpart on the right side.

To this point the development of the serrations of the leaves is also variable. In figure 6 they are slightly apparent on the edges bordering the cleft. In figure 7 they appear on the edges of both the periphery as well as the cleft, whereas in figure 8 they are absent from the periphery. The formation of the serrations occurs more often on the edges bordering the clefts than on the peripheral edge. This is to be expected since the periphery is destined to form the stipe and growing tip of the mature frond but it is of interest to point out that in several of the young stages the serrations are found in this region.

In figure 11 the development has progressed to the point where the secondary stems are well formed and pneumatocysts have developed on the bases of the leaves formed by the third cleft. In this stage there is also evidence of asymmetry noted above since one frond, the right in this case, is slightly in advance of the other. In this figure it is also to be pointed out that the primary cleft has not yet been completed and the tip of this plant is still essentially one blade.

In figure 12 the primary cleft has divided the plant into two halves, the pneumatocyst of the second leaf is well formed and the pneumatocyst of the third leaf is developing. Figures 13 and 14 of plants four to four and one-half months of age show the development of the first fronds, the elongation of the stipes and the establishment of the growing tip as well as the formation of the



Figures 1 to 15 inclusive

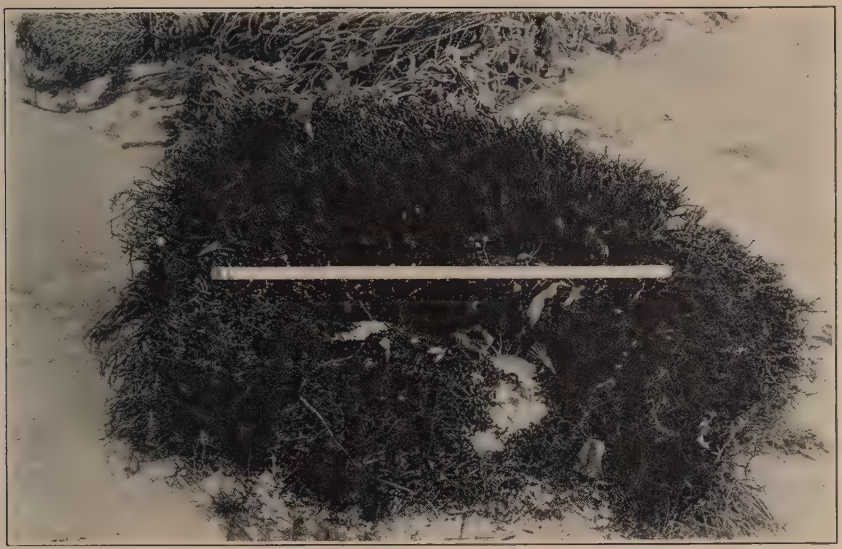


Figure 16

inner leaves which will subsequently give rise to secondary stipes and sporophylls. It is necessary to point out that the mechanics of preparing the specimens have ruptured the spherical cysts so that they have a distorted appearance. In nature they are hollow, thin-walled structures which even in these early stages are fairly resistant to pressure.

Figure 15, representing a plant of approximately five months of age, shows the appearance of the first cleft in the inner leaves (see arrow). This will establish the beginning of the second stipe which will repeat the process described and also form the first sporophyll.

The question of size attained by these plants is still widely discussed. Extreme lengths of several hundred to one thousand feet have been reported and these statements have appeared in texts. Frye, Riggs and Crandall have discussed this adequately in their publication. The observations of the writer tend to confirm their report of the size attained by these plants. Certainly none longer than 150 feet has been called to the attention of the writer.

However, figure 16, shows a holdfast which is in excess of the maximum reported by Setchell and Gardner for Macrocystis. The three foot rule laying on this specimen affords a means of comparison. By actual measurement this holdfast was 4.75 ft. in diameter. It is impossible to know the age of this plant but it is believed that it was at least five to eight years of age before it was torn loose by a storm and deposited on the beach. At the same time this plant was found, there were five others nearby all of which had holdfasts in excess of four feet.

Summary

1. A description is given of the development of the sporophyte of *Macrocystis pyrifera.*

2. A report is made of a holdfast of *M. pyrifera* 4.75 feet in diameter considerably in excess of the previously reported maximum size of this structure.

LITERATURE CITED

Brandt, R. P.
Early development and Growth of the Giant Kelp, Macrocystis pyrifera. U. S. D. A. Dept. Bull., No. 1191, 1923.

Frye, T. C., Riggs, G. B. and Crandall, W. C.
The Size of Kelps on the Pacific Coast of North America. Bot. Gaz., vol. 60, no. 6, pp. 473-482, 1915.

Setchell, William Albert
Macrocystis and its holdfasts. Univ. Calif. Pub. Bot., vol. 16, no. 13, pp. 445-492, plates 33-48, 1932.

Setchell, William Albert and Nathaniel Lyon Gardner
The Marine Algae of the Pacific Coast of North America. Part III Melanophyceae. Univ. Calif. Pub. Bot., vol. 8, pt. 3, pp. 626-628, plates 64 and 65, 1925.

Skottsberg, Carl
Zur Kenntnis der Subanarktischen und Antarktischen Meeresalgen I. Phaeophyceen. Wiss. Erg. Schwed. Südpolar-Exp. 1901-1903, Bd. IV, Lief. 6, z 80-136.

Contribution No. 179

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No. 2495, pages 379–380.

THE BACTERIAL OXIDATION OF RUBBER[1]

IN studying the biological oxygen demand of sea water[2] it has been observed that rubber stoppers increase the amount of oxygen consumed. In fact, the small amount of rubber gasket exposed to the water in citrate of magnesia bottles perceptibly increases the amount of oxygen consumed after 5 to 10 days incubation. Heat-sterilized, as well as formaldehyde-preserved, controls prove that the increased oxygen consumption is attributable to biological activity.

One-gram quantities of various kinds of rubber cut in small pieces to give a surface area of approximately 10 sq. cm were placed in 160 ml glass-stoppered bottles. After sterilizing in the autoclave at 120° C. for 20 minutes the bottles were filled with sterile water saturated with oxygen. Half of the bottles were inoculated and the others remained sterile. Duplicate bottles were analyzed for oxygen immediately using the Winkler technique, and the others were incubated in the water bath for one to five days at 22° C. after which the amount of oxygen consumed was determined. It is necessary to decant or siphon the water from the rubber before treating it with the Winkler reagents because free iodine reacts with rubber.

Using samples of rubber stoppers, rubber tubing, pure gum rubber, duprene and neoprene it was found that whereas 0.53 to 0.88 mgm/l of oxygen was consumed in the sterile controls after 5 days incubation, 5.11 to 6.74 mgm/l of oxygen was consumed in the inoculated water. Similar results were obtained when the controls were preserved with 1.0 per cent. for-

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 179.

[2] C. E. ZoBell, *Jour. Mar. Res.*, 3: 211, 1940.

maldehyde. That the increased oxygen consumption is caused by microorganisms is indicated by the fact that the rate of oxygen consumption increases exponentially with time typical of a growth-curve and, more convincingly, by the multiplication of microorganisms.

Realizing that most of the aforementioned rubber products contain sulphur and other oxidizable constituents used as fillers or impurities, the experiments were repeated with several samples of highly purified (non-vulcanized) caoutchouc or latex obtained from the Goodyear Tire and Rubber Company. Small quantities dissolved in C.P. benzene were distributed in bottles which were manipulated to form a thin film of the purified rubber on the inside of the bottles as the benzene evaporated. After driving off all the benzene the bottles were filled with oxygenated water. Following the incubation of inoculated water it was found that 2 to 2.5 mgm of oxygen was consumed in the presence of 1 mgm of purified rubber. This together with the production of carbon dioxide which was estimated manometrically indicated that most of the rubber was oxidized. Considering rubber to be $(C_5H_8)_x$, it would require about 3.3 mgm of oxygen to completely oxidize 1 mgm of rubber. Part of the rubber was converted into bacterial protoplasm as shown by the number of bacteria which appeared. The perforation of thin films of rubber on agar and on glass slides immersed in culture solutions gives further proof that rubber is utilized by microorganisms.

Confirming the observations of Söhngen and Fol[3] and Spence and van Niel[4] most of the rubber oxidizing microorganisms which have been observed belong

[3] N. L. Söhngen and J. G. Fol, *Centralbl. f. Bakt.*, II. Abt., 40: 87, 1914.

[4] D. Spence and C. B. van Niel, *Ind. and Eng. Chem.*, 28: 849, 1936.

to the genus *Actinomyces* or *Proactinomyces*. Many of the latter utilize complex hydrocarbons according to Umbreit.[5] Rubber oxidizing *Mycobacterium* and *Pseudomonas* have also been isolated from our enrichment cultures. The aerial mycelium of an unidentified mold virtually enveloped the moist rubber on which it was growing and its substrate mycelium seemed to penetrate the rubber.

Using oxygen consumption with adequate controls as a criterion of their presence, rubber oxidizing bacteria have been found to be quite widely distributed in the sea and in garden soil. Neither in nature nor in the laboratory do the rubber oxidizing microorganisms appear to require rubber or related hydrocarbons for their multiplicaion but old pieces of rubber hose and the cracks in tires found in moist places have proved to be good sources of cultures.

Besides showing that neither synthetic nor pure India rubber is biologically inert and proving that rubber oxidizing microorganisms occur fairly abundantly in nature, the observations suggest that such microorganisms may play an important role in the deterioration of rubber products. The life of rubber products which come in contact with moisture may be prolonged if ways can be found to retard or prevent the activity of rubber oxidizing microorganisms.

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[5] W. W. Umbreit, *Jour. Bact.*, 38: 73, 1939.

[6] On sabbatical leave from Brooklyn College, New York. Assisted by grant No. 555 from the American Philosophical Society.

Contribution No. 180

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OCCURRENCES OF "RED WATER" NEAR SAN DIEGO[1]

SINCE 1917 the Scripps Institution of Oceanography has given considerable attention to the phenomenon of "red water" and to the conditions of its occurrence. Although the information available concerning it is not much greater in detail in 1942 than it was at the time of previous reports,[2] certain aspects of the conditions appear to be growing more distinct.

1942 was characterized by two periods of "red water," although neither was so conspicuous as in one or two former years. The responsible organisms (dinoflagellates) differed at the two periods in 1942 in contrast to the fact that in the other years only one organism attained "red water" prominence in the year in and near La Jolla Bay. *Prorocentrum micans* Ehr. contributed the color in May, but *Goniaulax polyedra* Stein was causative in September. The duration of noticeable discoloration of the sea in May was about one week, in September about three weeks. The largest abundance recorded in May was 500,000 cells per liter, but probably streaks and patches of more than a million per liter were present. In September the numbers in routine catches at the Scripps Institution pier yielded a maximum of 1,000,000 cells per liter, but special catches showed abundances up to about 2,000,000 cells per liter.

In both May and September the conspicuous populations appeared to drift into the bay from the west. In some other years the invasion was from the northwest. However, in all years the evidence available clearly indicates origins outside of the local area. In 1924 the discoloration caused by *Prorocentrum* was first discovered by the institution boat at about ten miles directly off shore and it was not for several days that the appearance was distinct in La Jolla Bay. Differences in direction and speed of approach to La

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 180.

[2] W. E. Allen, SCIENCE, 78: 12–13, 1933; SCIENCE, 88: 55–56, 1938.

Jolla, considered in connection with the fact that there is no recognizable "nursery area" in the region, indicate rather strongly that growth of these populations to "red water" prominence is dependent upon conditions affecting particular masses of water rather than upon conditions affecting particular localized geographic areas.

Perhaps the most striking evidence concerning occurrences of "red water" acquired by the Scripps Institution in twenty-five years is that indicating zonation of conspicuous abundances. Most of this has come to hand since the institution began intensive operations at sea in 1938. From these and from certain earlier observations off shore, it appears certain that high abundance in Southern California does not occur as much as twenty-five miles from shore (possibly not more than fifteen) and that it does not reach to a depth of more than thirty meters (except in very rare instances). By way of contrast, the planktonic diatoms, which usually thrive under conditions apparently favorable to the planktonic dinoflagellates, have shown large populations far from shore, a hundred miles and more.

Here, of course, we have introduced a nice complexity of problems for marine hydrographers and chemists, etc., no less than for marine biologists. How can we account for such definite zonation with water boundaries of one and not the other of two groups closely associated? Still more difficult, how can we account for the fact that within these zones the "red water" organisms may show little prominence for years and then "suddenly" become conspicuous almost over night? Larger and smaller movements of water masses have a very definite place in the results, as do air conditions also, and there must be a long series of chemical and biological influences to run parallel with these when enormous development of numbers occurs. Until we know more about a number of these things our explanations of occurrences of "red water" must remain rather hazy except for some interesting generalities.

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Contribution No. 181

JOURNAL OF SEDIMENTARY PETROLOGY, VOL. 12, NO. 3, PP. 127–136,
FIG. 1, TABLE 1, DECEMBER, 1942

CHANGES PRODUCED BY MICROORGANISMS IN SEDIMENTS AFTER DEPOSITION*

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ABSTRACT

Significant numbers of bacteria have been found in recent sediments wherever critical tests have been made for their presence. Total numbers as well as the kinds of microorganisms decrease with core depth. Several physiological types of bacteria together with fewer yeasts, molds and actinomyces have been found, some at depths exceeding 15 feet. The demonstrated ability of the microorganisms to function in sedimentary materials in the laboratory under conditions simulating those in nature indicates that they could be active *in situ*, and changes in certain properties of sediments suggest that the microorganisms have been active.

Bacteria consume oxygen, and there are enough bacteria in sediments to account for the depletion of the dissolved oxygen. Similarly bacteria are probably responsible for the oxidation-reduction potential of sediments which ranges from Eh 0 to −0.5 volts whereas the overlying water is oxidizing in character. There are several ways in which the biochemical activities of microorganisms influence the hydrogen-ion concentration of sediments. Most types of organic matter are attacked by bacteria. The organic content and microorganisms influence the aggregation of particles. By altering the environmental conditions and by serving as a source of food, bacteria have a pronounced effect on the animal population of sediments.

It is still indeterminate if bacterial activity plays a role in the genesis of petroleum, but there are several ways in which it might. The changes in the calcium, iron, manganese, sulfur, phosphate and carbonate content of sediments attributable to microorganisms are mentioned.

INTRODUCTION

There are several lines of evidence which indicate that bacteria and allied microorganisms influence the diagenesis of sedimentary materials. (1) Significant numbers of living bacteria are found in recent sediments wherever critical tests have been made for their presence. (2) Several kinds of bacteria capable of altering various chemical substances or physico-chemical conditions occur in the sediments. (3) Controlled experiments in the laboratory show that the bacteria could be functional *in situ*, or in the environment in which they are found. (4) There is a correlation between the numbers and kinds of bacteria and the chemical and physico-chemical conditions found in the sediments.

BACTERIAL POPULATION

A few hundred to several million viable bacteria per gram of marine mud have been demonstrated by cultural procedures. The samples of mud have been collected and analyzed by methods which preclude the possibilities of extraneous contamination. Moreover, since there are far more bacteria in the mud than in the overlying water, and since the bacteria found in the mud are different from those found in other environments, there can be no question concerning the existence of bacteria in the mud.

In recent work, the coring instrument described by Emery and Dietz (1941) was used for the collection of mud samples for bacteriological analysis. The core barrel of the instrument (fig. 1) is lined with a long strip of 15-guage celluloid which facilitates the removal and examination of the mud core without unduly disrupting the natural stratification. With this instrument numerous cores ranging in length from 6 feet to a maximum length of nearly 17 feet have been taken from many types of sediments

* Contribution from the Scripps Institution of Oceanography, New Series No. 181.

from different depths of water down to 12,200 feet. In earlier work we have used a modified Ekman mud-sampling device, and the Trask coring tube has been used.

Radially central portions of the core samples have been dissected out aseptically and transferred to sterile tared

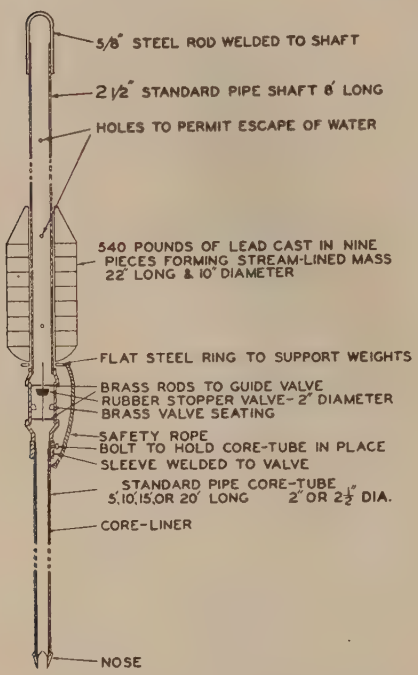
5/8" STEEL ROD WELDED TO SHAFT
2 1/2" STANDARD PIPE SHAFT 8' LONG
HOLES TO PERMIT ESCAPE OF WATER
540 POUNDS OF LEAD CAST IN NINE PIECES FORMING STREAM-LINED MASS 22" LONG & 10" DIAMETER
FLAT STEEL RING TO SUPPORT WEIGHTS
BRASS RODS TO GUIDE VALVE
RUBBER STOPPER VALVE- 2" DIAMETER
BRASS VALVE SEATING
SAFETY ROPE
BOLT TO HOLD CORE-TUBE IN PLACE
SLEEVE WELDED TO VALVE
STANDARD PIPE CORE-TUBE 5,10,15,OR 20' LONG 2" OR 2½" DIA.
CORE-LINER
NOSE

FIG. 1.—Coring device described by Emery and Dietz (1941).

bottles in which the weight of the samples could be ascertained. The samples were mixed and diluted with sterile sea water after which appropriate dilutions were planted on plates of nutrient sea water agar or in selective media designed to promote the growth of different physiological types of bacteria. The plate-count and minimum dilution procedures give information on the numbers and kinds of bacteria in the mud, and from the media pure cultures can be isolated for further study.

In nearly all cores examined the topmost portions, or the layer of mud in immediate contact with the overlying water, showed the largest bacterial populations. In the topmost 2-inch layer of mud carefully collected manually in shallow waters of Mission Bay, ZoBell and Feltham (1942) reported the presence of a maximum of 460,000,000 bacteria per gram of sedimentary material (dry basis). In different marine environments the number of bacteria in the surface layers of sediment ranges from thousands to hundreds of millions. Among the factors which influence the number of bacteria, the presence of available organic matter seems to be the most important (Henrici and McCoy, 1938, Williams and McCoy, 1935, Waksman et al., 1933, Waksman and Hotchkiss, 1938, ZoBell and Anderson, 1936). The particle size is also an important factor, there generally being far more bacteria in fine and colloidal sediments than in coarse ones (ZoBell, 1938).

With increasing core depth the bacterial population decreases sharply (Rittenberg, 1940). The decrease is most rapid in the first few inches after which it is comparatively slow. This is illustrated by the data in table 1 which shows the number of bacteria found at different depths of some cores from off the coast of Southern California. At depths exceeding 10 inches the decrease is gradual but more or less sporadic. In most cores fewer than ten thousand living bacteria per gram are found at depths below 10 inches, although occasionally a stratum of sediment containing millions of bacteria per gram is found at great depths. These bacteria-rich strata differ also in other characteristics which suggests that the bacteria are active *in situ*.

Bacteria do not contribute much to the mass of sediments, more than a billion cells being required to give a mass of one milligram. However, the bacteria may produce significant quantities of biocatalysts or enzymes which activate a large variety of chemical reactions in the sediments. Some of these enzymes may continue to be functional after the bac-

teria themselves are dead. According to Kreps (1934) organic catalysts or enzymes are concentrated in bottom deposits and the observations of ZoBell (1939) show that at least certain types of enzymes continue to be active in sediments in the absence of bacteria.

KINDS OF MICROORGANISMS

The majority of microorganisms recovered from sedimentary materials prove to be bacteria although actinomyces, yeasts, molds and algae have been noted. Sparrow (1937) has isolated 18 species representing 8 genera of saprophytic fungi from marine mud where they were found associated with decaying plankton. Blue-green algae and diatoms are commonly found on the bottoms of shallow lakes and seas but their activity is probably limited by the depth to which sunlight penetrates. Actinomyces (Umbreit and McCoy, 1941) appear to be fairly common in sedimentary materials.

The bacteria isolated from bottom deposits are biochemically versatile, being endowed with the ability to attack several inorganic substances and most kinds of organic matter. Bacteria are found in nearly all samples which reduce nitrate to nitrite, free nitrogen or ammonia. Rittenberg (1941) found *Vibrio desulfuricans*, an organism which reduces sulfate to hydrogen sulfide and possibly to free sulfur, in 44 per cent of the 450 samples of marine sediments from 160 localities and depths down to 16 feet below the sea floor. *Vibrio desulfuricans* appears to be active *in situ*, thereby influencing chemical and geological conditions.

Sediments from fresh-water lakes (Williams and McCoy, 1935) as well as marine sediments (ZoBell, 1938) contain an abundance of bacteria which decompose proteins with the formation of carbon dioxide, ammonium, hydrogen sulfide and a variety of intermediate products. Many kinds of carbohydrates are converted into carbon dioxide, carbon monoxide, methane and organic acids. Most lipins or fats which have been tested are hydrolyzed with the formation of fatty acids. The glycerol liberated when fats are hydrolyzed is oxidized by most bacteria and some of them utilize the fatty acids also. It is especially noteworthy that complex organic substances including chitin, spongin, waxes, hydrocarbons of varying degrees of complexity (Grant and ZoBell, 1942), cellulose, hemicellu-

TABLE 1. *Number of Bacteria Found per Gram of Wet Marine Sediments in Different Strata of Cores*

Core number Station location Water depth	XIV-37 32°26.4′N. 117°41.3′W. 3120 feet	XIV-45 32°36.4′N. 117°27.8′W. 3570 feet	XIV-53 33°03.3′N. 117°25.5′W. 1415 feet
Core depth in inches	Bacteria per gram	Bacteria per gram	Bacteria per gram
0–1	38,000,000	7,500,000	840,000
1–2	940,000	250,000	102,000
4–5	88,000	160,000	63,000
9–10	36,000	23,000	19,000
14–15	2,400	8,700	1,500
19–20	400	2,100	2,200
29–30	180	600	370
39–40	330	200	190
59–60	250	300	210
79–80	130	100	140
99–100	290	150	140

lose, agar (Bavendamm, 1931) and lignin (ZoBell and Stadler, 1940) are decomposed by certain mud-dwelling bacteria.

The aforementioned bacteria are heterotrophs requiring organic matter as a source of carbon or energy. Autotrophs, or those which obtain their energy from the oxidation of inorganic substances such as ferrous iron, manganous manganese, hydrogen sulfide, hydrogen, carbon monoxide, methane or ammonium as a source of energy, have likewise been found in recent sediments. If they are active *in situ* the autotrophs may be of importance in transforming inorganic substrates and in synthesizing organic matter (bacterial protoplasm) from carbon dioxide, water and minerals. It is doubtful, though, if the autotrophs are active except at the mud-water interface because most of them are aerobes whose activity requires free oxygen, and, as will be elaborated below, there is little or no free oxygen in sub-surface sediments.

EVIDENCE OF ACTIVITY

Merely finding microorganisms in sedimentary materials fails to prove that they are biochemically active in such an environment. Algae, diatoms, and other photosynthetic organisms sometimes found in sediments, obviously are not active below the zones penetrated by light. Similarly it is quite likely that many of the bacteria found in sediments are in a resting or inactive state. For example, the strict aerobes (the bacteria whose activity requires free oxygen) could not be active in the reduced sediments containing no oxygen from which they have been recovered. However, controlled laboratory experiments designed to simulate the marine environment as well as conditions found in the sediments indicate that many of the bacteria occurring in sedimentary materials are active *in situ*.

ZoBell (1934) has summarized the literature which shows that mud-dwelling bacteria multiply and are otherwise physiologically active at temperatures as low as any found on the sea floor (0 to −4° C.). They are not inhibited by the osmotic pressure of sea water nor by the high hydrostatic pressures encountered at great depth. Similarly, the bacteria thrive in an environment which is as oxidizing (Eh +0.08 volts) or as reducing (Eh −0.51 volts) as any which has been found in bottom deposits. Finally and most conclusive, the bacteria multiply and exhibit other manifestations of biochemical activity in samples of sedimentary materials from different depths and stations kept under observation in the laboratory.

OXYGEN UTILIZATION

When stored in the laboratory the bacterial population of freshly collected mud samples may increase from a ten- to a thousand-fold, the rate and magnitude of the increase being largely a function of the temperature. The storage is accompanied by changes in the organic matter content, pH, oxidation-reduction potential and hydrogen sulfide content. If the mud is mixed with sea water, dissolved oxygen is depleted from the latter. ZoBell (1939) has reported that muds from the Pacific Ocean consume from 0.5 to 3 cc. of oxygen per gram (dry basis), the exact amount being a function of the quantity and quality of organic matter present and the time and temperature of incubation. Anderson (1940) analyzed 131 samples of marine mud from the Cape Cod region and found that from 2.2 to 1000 cc. of oxygen was consumed by bacteria for each gram of organic carbon present; the organic carbon content of the muds ranging from 0.11 to 4.5 per cent of the sample.

Indications are that the rate of bacterial activity in marine sediments is very slow, particularly in deep water or at high latitudes where temperatures are low. The temperature range of approximately 80 per cent of the ocean floor is between 0 to 5°C. ZoBell (1940) has found that at this temperature marine bacteria consume an average of only 1.9×10^{-12} cc. of oxygen per cell per hour. At this rate it would require about ten million bacteria to utilize 1.0 cc. of oxygen in a year.

However, ten to a hundred million bacteria are often found in a gram of surface sedimentary material, which even at low temperatures is enough to account for the depletion of the dissolved oxygen from the interstitial water. In many localities the dissolved oxygen is consumed also from the immediately overlying water faster than it can be replaced by diffusion from above. In the shoal waters of Mission Bay, ZoBell and Feltham (1942) estimated that there were potentially enough bacteria in a square meter of mud to a depth of 1.0 cm. to consume from 6 to 18 cc. of oxygen per day. Actually they found that there was no free oxygen in the sub-surface layers of mud, and the oxygen tension of the overlying water was reduced.

Rarely is there the slightest trace of dissolved oxygen in the interstitial water of marine sedimentary materials. Therefore, it is believed that one of the first changes caused by bacterial activity is the depletion of oxygen, thereby rendering the environment anaerobic.

OXIDATION-REDUCTION POTENTIAL

After the oxygen is quantitatively consumed by bacteria, they continue to function at the expense of other hydrogen acceptors in the sedimentary materials as indicated by the reducing conditions, or low oxidation-reduction potential (Hewitt, 1936), created. This has been demonstrated in the laboratory as well as by measuring the oxidation-reduction potential of freshly collected sediments from different core depths. Several hundred electrometric determinations show that as a rule the oxidation-reduction potential of sediments decreases with core depth throughout the topmost 25 to 50 cm. from Eh 0, or slightly positive values, at the mud-water interface to Eh −0.4 volts. At greater depths the results have been erratic, although reducing conditions (Eh −0.2 to −0.5 volts) usually prevail. The overlying water is generally oxidizing in character (Allgeier et al., 1941).

The erratic results are attributed partly to technical difficulties in making the measurements and partly to the decrease in the content of reversibly oxidizable or reducible constituents at depths exceeding 25 to 50 cm. below the mud-water interface. The redox or poising capacity, as indicated by the amount of reducing substances in the sediments, usually decreases with core depth. This has been determined by noting the oxygen-absorbing capacity of samples. The redox capacity has also been determined from the amount of reducible dye such as methylene blue, for example, which is reduced by known quantities of mud.

The oxidation-reduction potential has a marked influence upon the direction, velocity and magnitude of chemical reactions which involve the exchange of electrons, or oxidation or reduction of reactants. Consequently, the oxidation-reduction potential would be expected to influence the diagenesis of sedimentary materials. Besides being related to the oxygen tension, Pearsall and Mortimer (1939) find that the state of iron, sulfur and certain nitrogen compounds is a function of the oxidation-reduction potential. According to Brujewicz (1937) the state of manganese, as well as iron, is a factor of the oxidation-reduction potential of marine sediments. Allgeier et al. (1941) find that the principal effect of the oxidation-reduction potential of freshwater lake deposits is on the content of ferrous iron, hydrogen sulfide and organic matter.

HYDROGEN-ION CONCENTRATION

Bacterial activity has the tendency to change the hydrogen-ion concentration or pH of sedimentary materials. Depending upon the numbers and kinds of microorganisms present, the substrate and environment conditions, they might increase the acidity or decrease it. The hydrogen-ion concentration is increased by the production of organic acids, including carbonic acid, from the decomposition of organic matter, by the formation of sulfate or nitrate, or by the utilization of ammonium. The hydrogen-ion con-

centration may be decreased by the reduction of sulfate or nitrate, by the utilization of organic acids, or by the formation of ammonium from nitrogenous compounds.

Bacteria capable of activating any one of the aforementioned reactions have been demonstrated in sedimentary materials. This, together with changes observed to occur under laboratory conditions, leads to the conclusion that bacteria are the principal dynamic agents which are responsible for changes in the hydrogen-ion concentration in sedimentary materials. The hydrogen-ion concentration of bottom deposits at depths to which carbon dioxide does not diffuse from the atmosphere is probably largely a function of the interrelated factors which influence the abundance, kinds and activity of bacteria. An appraisal of these factors will require much more quantitative information. According to Miyadi (1934), bacterial activity has a pronounced effect upon the hydrogen-ion concentration of lake mud, and Kusnetzow (1935) reports that bacterial activity may affect the hydrogen-ion concentration as well as the oxidation-reduction potential of an entire water basin.

Besides influencing the solubility and reactivity of certain constituents of sediments, the hydrogen-ion concentration also influences the oxidation-reduction potential. Its effects on base exchange and on the properties of clay have been discussed by Kelley (1939) and Grim (1939).

The hydrogen-ion concentration of the topmost layers of bottom deposits is usually nearly the same as that of the overlying water. For sea water it ranges from pH 7.6 to 8.3, depending primarily upon the depth of the overlying water and its origin. In some cores from the Gulf of California and the Channel Island region off the coast of Southern California the pH increases with core depth to as much as pH 8.9, and in other cores it decreases with depth to as low as pH 7.2. In freshwater lakes the range is far greater (Allgeier et al., 1941), varying considerably from lake to lake.

The unpublished observations of Rittenberg and Revelle at the Scripps Institution of Oceanography indicate that the pH of marine sediments is more related to particle size than to core depth. The highest pH values which are found usually occur in sediments in which most of the sulfate in the interstitial water has been reduced, presumably by bacterial activity. *Vibrio desulfuricans*, an anaerobic bacterium which reduces sulfate, has been found to be quite widely distributed in these sediments.

ORGANIC MATTER

An appreciable portion of the organic content of recent deposits may consist of fragments of various sizes of the bodies of animals and plants (Twenhofel, 1939). While some of this may be buried and preserved more or less intact in the sediments, the remains of most organisms are rapidly disintegrated by bacterial activity.

The majority, if not all, of the bacteria occurring in sediments require organic matter for building material and energy. The gradual decrease with core depth of the organic content of bottom sediments gives further evidence that bacteria have been active since their deposition. In general, the changes in the quality as well as in the quantity of organic matter in samples of sedimentary materials with core depth are greatest where bacteria are most abundant, namely, in the topmost few inches of mud (Trask, 1932).

The total quantity of organic matter decreases 10 to 20 per cent in the first two or three inches, and a like amount in the next 2 or 3 feet. At core depths exceeding 2 or 3 feet changes in the organic content of sediments is almost imperceptible as a rule. The work of Anderson (1940) shows that the organic matter becomes more refractory to bacterial oxidation with core depth, probably because the most oxidizable fractions are oxidized first leaving the so-

called humus (Waksman, 1933). It is estimated that between 30 and 40 per cent of the organic matter which is utilized by bacteria is converted into bacterial protoplasm which is fairly complex. Possibly a large percentage of the humus and other residual organic matter in older sedimentary material consists of bacterial cells or the remains thereof.

If it is permissible to generalize in terms of what our fragmentary observations indicate are mean conditions, it can be pointed out that in sediments taken from depths exceeding 2 or 3 feet, the order of magnitude of the bacterial population is thousands per gram. Throughout a foot of sediments from this depth the organic content has been found to decrease approximately 1.0 mgm. per gram of sediment. Assuming that uniformitarianism has prevailed and that the bacteria utilize organic matter at the rate of 10×10^{-12} mgm. per cell per hour (an approximation based upon laboratory observations), it would require around 10,000 years for the bacteria in one gram of such mud to oxidize 1.0 mgm. of organic matter. According to Trask (1939) sediments from this region have been deposited at the rate of around one foot in 10,000 years. While allowances must be made for errors in arriving at these estimates, it is significant that the order of magnitude of the estimates made by the bacteriologists approximate those made by the geologists.

BINDING ACTION

Besides influencing the texture and composition of sedimentary materials, the organic content and especially bacterial cells have a tendency to cement particles together thereby influencing the lithification of sediments (Shrikhande, 1936, and Peele, 1940). Many bacteria are extremely adhesive, growing tenaciously attached to solid surfaces, and some of them produce mucilaginous exudates. Filamentous cells also have a binding action on the aggregation of particles. Martin and Waksman (1940) report that microorganisms have a marked binding action on soil particles, the extent of the binding depending primarily upon the types of organisms and the quality of the organic matter present. While there may not be enough living cells to have any appreciable binding effect, there are probably thousands of times as many dead and partly decomposed bacterial cells as the number of living cells which can be demonstrated.

EFFECT ON OTHER ORGANISMS

As pointed out by Twenhofel (1939) most sediments after deposition contain plant and animal matter which serves as a source of food for numerous mud-eating, burrowing and browsing animals. Such animals have far-reaching effects on the diagenesis of sedimentary materials, and in turn bacteria influence the distribution and activity of such animals. By depleting the dissolved oxygen content, by reducing the oxidation-reduction potential, by producing hydrogen sulfide and other toxic products, and by increasing the hydrogen-ion concentration, bacteria create environmental conditions in certain localities which are inimical to other forms of life. No burrowing animals are found in black stinking muds from which bacteria have consumed all of the oxygen and produced large quantities of hydrogen sulfide.

On the other hand bacteria may be beneficial by serving as an important source of food for mud-dwelling animals and by mineralizing organic matter with the production of plant nutrients. It has been demonstrated by Baier (1935), MacGinitie (1935) and ZoBell and Feltham (1938) that many mud-dwelling animals are nourished largely by bacteria which they ingest and digest. The bacteria assimilate dissolved organic matter as well as lignin, spongin, chitin, cellulose and other complex compounds which are unfit as food for most types of animals which can utilize the bacteria.

The bacterial flora of a marine mud flat as an ecological factor has been stud-

ied by ZoBell and Feltham (1942). They conclude that the numbers and kinds of mud-burrowing animals as well as the depths to which the animals penetrate are influenced by environmental conditions produced by bacteria and allied microorganisms.

PETROLEUM GENESIS

It is still indeterminate if bacterial activity plays a direct role in the formation of petroleum but the accumulating evidence is highly suggestive. In the transformation of organic matter, bacteria convert it into substances which are more petroleum-like by reducing its nitrogen, oxygen, phosphorus and sulfur content (see analyses summarized by ZoBell, 1942). The low oxidation-reduction potentials which bacteria create in certain environments are conducive theoretically to the formation and accumulation of hydrocarbons. Lipolytic bacteria which hydrolyze fats with the liberation of fatty acids occur in sediments. It would be necessary only to remove two atoms of oxygen from these fatty acids to give fairly long-chain hydrocarbons, but to date bacteria which do this have not been found. Methane-producing bacteria are widely distributed in sedimentary materials but bacteria which produce higher hydrocarbons, with the exception of complex polyene pigments and their allies (Fox and Anderson 1941), have not been demonstrated.

The unconfirmed report of Ginzburg-Karagicheva (1936) finding 93 different saturated and unsaturated hydrocarbons besides methane being produced by anaerobic bacteria and that of Sturm and Orlova (1937) of a liquid hydrocarbon fuel being produced anaerobically from fats cannot be ignored.

On the other hand, the work of Bushnell and Haas (1941), Grant and ZoBell (1942) and others shows that benzene, cyclohexane, toluene, xylene, naphthalene and several aliphatic hydrocarbons from methane up to paraffin and waxes are oxidized by certain bacteria. Is it possible that such bacteria destroy hydrocarbons as rapidly as they are produced in sedimentary materials except in certain source beds where conditions are not conducive to the activity of hydrocarbon-oxidizing bacteria? The answer to this question may have a bearing upon the explanation of petroleum formation and accumulation.

One popular theory which has been advanced to account for the origin of oil premises the presence of free hydrogen in the source beds. It is possible that the hydrogen could be produced by bacterial activity. Bacteria which produce hydrogen during the anaerobic decomposition of organic matter occur in bottom deposits at all depths which have been explored. Allgeier et al. (1932) noted that after prolonged incubation under anaerobic conditions up to 12 per cent of the organic content of lacustrine sediments was converted into gaseous products consisting of 65 to 85 per cent methane, 3 to 30 per cent carbon dioxide, 3 to 22 per cent nitrogen and 1 to 3 per cent hydrogen. We demonstrated appreciable quantities of hydrogen as well as methane in freshly collected sediments from the Gulf of California.

INORGANIC CONSTITUENTS

Many workers attribute the origin of sedimentary iron formations primarily to the activities of microorganisms. The direct and indirect ways in which bacteria influence the solution or precipitation of iron in sediments have been outlined by Starkey and Halvorson (1927) and Baier (1938). They also discuss the ways in which compounds of manganese are transformed by biological agencies.

Thiel (1928) points out that bacteria are agents of rock decomposition. When bacteria are present in percolating water 50 per cent more mineral matter may be dissolved from igneous and sedimentary rocks than when bacteria are absent. Aluminum is liberated from hydrous aluminum silicates during rock decay by bacterial action. Potassium is leached from potassium bearing minerals due to its assimilation by bacteria and fungi.

Zinc blend is oxidized to zinc sulfate, and zinc carbonate and silicates are dissolved by the sulfuric acid formed by the bacterial oxidation of hydrogen sulfide and sulfur. There are numerous ways in which sulfur compounds are transformed by bacterial activity. Thiel (1930) reports that calcium phosphate as well as calcium carbonate is dissolved by bacteria which produce acid during the decomposition of organic matter.

If bacterial activity has any effect on the precipitation or subsequent dissolution of calcium carbonate, it is caused primarily by changes in the pH or hydrogen-ion concentration of the sediments or of the overlying water. In general, high pH values promote the precipitation of calcium carbonate while low pH values favor the dissolution of calcium carbonate.

The role of microorganisms in the precipitation of calcium carbonate has been summarized by Bavendamm (1932) and Baier (1937). According to Baier bacteria effect calcium carbonate precipitation primarily by their effect on the pH and secondarily by their effect on the carbon dioxide tension and organic matter content of sedimentary materials. To these could be added the indirect effect that bacterial activity may have on environmental conditions which influence other biological entities such as animals with calcareous remains and photo-synthetic organisms which are known to influence the availability and solubility of calcium.

REFERENCES

Allgeier, R. J., Peterson, W. H., Juday, C. and Birge, E. A., *1932*, The anaerobic fermentation of lake deposits: *Internat. Rev. d. ges. Hydrobiol. u. Hydrog.*, vol. 26, pp. 444–461.

———, Hafford, B. C., and Juday, C., *1941*, Oxidation-reduction potentials and pH of lake waters and lake sediments: *Trans. Wis. Acad. Sci.*, vol. 33, pp. 115–133.

Anderson, D. Q., *1940*, Distribution of organic matter in marine sediments and its availability to further decomposition: *Jour. Mar. Res.*, vol. 2, pp. 225–235.

Baier, C. R., *1935*, Studien zur Hydrobakteriologie stehender Binnengewasser: *Archiv. f. Hydrobiol.*, vol. 29, pp. 183–264.

———, *1937*, Die Bedeutung der Bakterien für den Kalktransport in den Gewässern: *Geol. d. Meere u. Binnengewässern*, vol. 1, pp. 75–105.

———, *1938*, Die Bedeutung der Bakterien für die Bildung oxydischer Eisen- und Manganerze. *ibid.*, pp. 325–48.

Bavendamm, W., *1931*, Die Zersetzung der Hemizellulosen, besonders von Agar-Agar durch das meeresbakterium Bacillus gelaticus Gran: *Ber. Deutsch. Bot. Ges.*, vol. 49, pp. 288–290.

———, *1932*, Die mikrobiologische Kalkfällung in der tropischen See: *Arch. f. Mikrobiol.*, vol. 3, pp. 205–76.

Brujewicz, S. W., *1937*, Oxidation-reduction potentials and pH of sea bottom deposits: *Trav. de l'Assoc. Internat. de Limnol. theor. et appl.*, vol. 8, pp. 35–49.

Bushnell, L. D., and Haas, H. F., *1941*, The utilization of certain hydrocarbons by microorganisms: *Jour. Bact.*, vol. 41, pp. 653–673.

Emery, K. O., and Dietz, R. S., *1941*, Gravity coring instrument and mechanics of sediment coring: *Geol. Soc. Amer., Bull.*, vol. 52, pp. 1685–1714.

Fox, D. L. and Anderson, L. J., *1941*, Pigments from marine muds: *Nat. Acad. Sci.*, vol. 27, pp. 333–336.

Ginsburg-Karagitscheva, T. L., *1933*, Microflora of oil waters and oil-bearing formations and biochemical processes caused by it: *Amer. Assoc. Petrol. Geol., Bull.*, vol. 17, pp. 52–65.

Grant, C. W. and ZoBell, C. E., *1942*, Oxidation of hydrocarbons by marine bacteria: *Proc. Soc. Exper. Biol. & Med.* (in press).

Grim, R. E., *1939*, Properties of clay: *Recent Marine Sediments*. Amer. Assoc. Petrol. Geol., Tulsa, Okla., pp. 466–495.

Henrici, A. T. and McCoy, Elizabeth, *1938*, The distribution of heterotrophic bacteria in the bottom of some lakes: *Trans. Wis. Acad. Sci.*, vol. 31, pp. 323–361.

Hewitt, L. F., *1936*, Oxidation-reduction potentials in bacteriology and biochemistry: *London County Council*, pp. 1–101.

Kelley, W. P., *1939*, Base exchange in relation to sediments: *Recent Marine Sediments*. Amer. Assoc. Petrol. Geol., Tulsa, Okla., pp. 454–465.

Kreps, E., *1934*, Organic catalysts or enzymes in sea water: *James Johnstone Memorial Volume, Liverpool Univ. Press*, pp. 193–202.

KUSNETZOW, S. I., *1935*, Microbiological researches in the study of the oxygen regimen of lakes: *Verhandlungen der Internat. Ver. f. Theor. und Angen. Limnologie*, vol. 7, pp. 562–582.
MACGINITIE, G. E., *1935*, Ecological aspects of a California marine estuary: *Amer. Midland Naturalist*, vol. 16, pp. 629–765.
MARTIN, J. P. and WAKSMAN, S. A., *1940*, Influence of microorganisms on soil aggregation and erosion: *Soil. Sci.*, vol. 50, pp. 29–47.
MIYADI, D., *1934*, Oxygen absorption of the lake deposit: *Proc. Imper. Acad., Kyoto Imper. Univ.*, vol. 10, pp. 236–239.
PEARSALL, W. H. and MORTIMER, C. H., *1939*, Oxidation-reduction potentials in water-logged soils, natural waters and muds: *Jour. Ecol.*, vol. 27, pp. 483–501.
PEELE, T. C., *1940*, Microbial activity in relation to soil aggregation: *Jour. Amer. Soc. Agron.*, vol. 32, pp. 204–212.
RITTENBERG, S. C., *1940*, Bacteriological analysis of some long cores of marine sediments: *Jour. Mar. Res.*, vol. 3, pp. 191–201.
———, *1941*, Studies on marine sulphate-reducing bacteria: *Dissertation, University of California*, pp. 1–96.
SHRIKHANDE, J. G., *1935*, The production of mucus during decomposition of plant materials: *Biochem. Jour.*, vol. 27, pp. 1551–1562. Also vol. 30, pp. 1789–1794.
SPARROW, F. K., *1937*, The occurrence of saprophytic fungi in marine muds: *Biol. Bull.*, vol. 73, pp. 242–248.
STARKEY, R. L. and HALVORSON, H. O., *1927*, Studies on the transformations of iron in nature. II. Concerning the importance of micro-organisms in the solution and precipitation of iron: *Soil Sci.*, vol. 24, pp. 381–402.
STURM, L. D. and ORLOVA, S. I., *1937*, On the transformation of fat, paraffin and palmitic acid under the influence of micro-organisms from the Ala-Kule Lake: *Microbiol.* (Russian), vol. 6, pp. 754–772.
THIEL, G. A., *1928*, A summary of the activities of bacterial agencies in sedimentation: *Nat. Res. Council Circular*, vol. 85, pp. 61–77.
———, *1930*, Recent studies on the influence of biochemical agencies in sedimentation: *Nat. Res. Counsel Bull.*, vol. 98, pp. 57–64.
TRASK, P. D., *1932*, Origin and Environment of Source Sediments of Petroleum: *Gulf Pub. Co., Houston, Texas*, 323 pp.
———, *1939*, Organic content of recent marine sediments: *Recent Marine Sediments*. Amer. Assoc. Petrol. Geol., Tulsa, Okla., pp. 428–453.
TWENHOFEL, W. H., *1939*, *Principles of Sedimentation*. McGraw Hill, New York, 610 pp.
UMBREIT, W. W. and MCCOY, ELIZABETH, *1941*, The occurrence of Actinomycetes of the genus Micromonospora in inland lakes: *Symposium on Hydrobiology*, Univ. of Wis., pp. 106–114.
WAKSMAN, S. A., *1933*, On the distribution of organic matter in the sea bottom and the chemical nature and origin of marine humus: *Soil Sci.*, vol. 36, pp. 125–147.
———, et al., *1933*, Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms: *Biol. Bull.*, vol. 64, pp. 183–205.
———, and HOTCHKISS, MARGARET, *1938*, On the oxidation of organic matter in marine sediments by bacteria: *Jour. Mar. Res.*, vol. 1, pp. 101–118.
WILLIAMS, F. T. and MCCOY, ELIZABETH, *1935*, The microflora of the mud deposits of Lake Mendota: *Jour. Sed. Petrology*, vol. 5, pp. 31–36.
ZOBELL, C. E., *1934*, Microbiological activities at low temperatures with particular reference to marine bacteria: *Quart. Rev. Biol.*, vol. 9, pp. 460–466.
———, *1938*, Studies on the bacterial flora of marine bottom sediments: *Jour. Sed. Petrology*, vol. 8, pp. 10–18.
———, *1939*, The occurrence and activity of bacteria in marine sediments: *Recent Marine Sediments*, Amer. Assoc. Petrol. Geol., Tulsa, Okla., pp. 416–427.
———, *1940*, The effect of oxygen tension on the rate of oxidation of organic matter in sea water by bacteria: *Jour. Mar. Res.*, vol. 3, pp. 211–223.
———, *1942*, Bacteria of the marine world: *Sci. Month.*, vol. 55, pp. 320–330.
———, and ANDERSON, D. Q., *1936*, Vertical distribution of bacteria in marine sediments: *Amer. Assoc. Petrol. Geol., Bull.*, vol. 20, pp. 258–269.
———, and FELTHAM, CATHARINE B., *1938*, Bacteria as food for certain marine invertebrates. *Jour. Mar. Res.*, vol. 1, pp. 312–327.
———, *1942*, The bacterial flora of a marine mud flat as an ecological factor. *Ecol.*, vol. 23, pp. 69–78.
———, and STADLER, JANICE, *1940*, The oxidation of lignin by lake bacteria: *Arch. f. Hydrobiol.*, vol. 37, pp. 163–171.

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Oxidation of Hydrocarbons by Marine Bacteria.*

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Using oxygen consumption as a criterion it has been demonstrated that various kinds of pure and mixed hydrocarbons are oxidized by bacteria found in sea water and marine mud. The bacteria were grown in sea water enriched with hydrocarbons as the only source of energy. In the initial experiments the manometric technic was used for following oxygen consumption but later it was found that more accurate results were obtainable by determining the dissolved oxygen content of water.

Sea water inoculated with enrichment cultures was thoroughly shaken to insure uniformity in its composition and its saturation with oxygen, after which it was siphoned into 150 ml glass-stoppered bottles. While holding the bottles at an angle to provide for trapping the hydrocarbon in the shoulder of the bottle, 0.1 ml of hydrocarbon was introduced with a pipette. The bottle was then stoppered, care being taken not to entrap any air bubbles. Duplicate bottles of water were analyzed at once for oxygen content using the Winkler technic, and other bottles were analyzed after different periods of incubation in a water bath at 22°C.

The protocol of a representative experiment in which raw sea water was used is summarized in Table I. Similar results were obtained with aged sea water which was virtually organic matter-free as indicated by the fact that its B.O.D. was less than 1.0 mg/l.

It is especially noteworthy that toluene which is sometimes used as a preservative of biological materials was attacked by certain marine bacteria. Soil bacteria which oxidize toluene have been described by Gray and Thornton[1] and by Tauson.[2] Incidentally in these studies we found marine microörganisms which utilize phenol and cresol. Some of them tolerate a saturated aqueous solution of tricresol and as much as 1.0% phenol.

Besides the hydrocarbons listed in Table I, we have noted the bacterial oxidation of petroleum ether, paraffin wax, paraffin oil, vaseline, benzene, xylene, n-hexane, pyridine and naphthalene as indicated by oxygen consumption and by bacterial multiplication. Enough bacteria develop in aged sea water enriched with certain hydrocarbons to render it turbid. Pronounced differences have been noted in the rate of utilization of different kinds of hydrocarbons but due to differences in solubilities and other technical difficulties, many factors which influence utilizability of the various classes of hydrocarbons are still obscure. In general, it appears that aliphatic hydrocarbons are attacked more readily than cyclic or aromatic compounds, and long chains are more susceptible to bacterial oxidation than hydrocarbons of small molecular weight.

All 60 ml samples of sea water and all 0.1 g samples of mud examined show the presence of bacteria which are able to utilize hydrocarbons. From enrichment cultures several species of *Proactinomyces*, *Pseudomonas* and *Mycobacterium* have been isolated. Bacteria which oxidize various kinds of petroleum hydrocarbons have been isolated from soil by Tauson,[2] Haas *et al.*,[3] Stone *et al.*[4] and others. The marine hydrocarbon-oxidizing bacteria differ greatly in their ability to attack different hydrocarbons.

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1 Gray, P. H. H., and Thornton, H. G., *Centralbl. f. Bakt.*, Abt. II, 1928, **73**, 74.

2 Tauson, V. O., *Planta*, 1929, **7**, 735.

3 Haas, H. F., Yantzi, M. F., and Bushnell, L. D., *Trans. Kansas Acad. Sci.*, 1941, **44**, 39.

4 Stone, R. W., Fenske, M. R., and White, A. G. C., *J. Bact.*, 1942, **44**, 169.

TABLE I.
Oxygen Consumed by Bacteria in Raw Sea Water Treated with Hydrocarbons.

Hydrocarbon	Initial dissolved O_2 content, mg/l	Dissolved O_2 after 14 days, mg/l	O_2 consumed 14 days, mg/l	O_2 consumed oxidizing hydrocarbon, mg/l
None (Control)	8.46	5.99	2.47	—
Crude oil	8.50	0.00	8.50	6.03
Petroleum ether	8.57	0.00	8.57	6.10
Kerosene	8.60	1.72	6.88	4.41
Gasoline	8.60	0.00	8.60	6.13
Toluene	8.64	0.00	8.64	6.17

They do not require hydrocarbons for their growth.

Marine hydrocarbon-oxidizing bacteria may influence the formation, accumulation and storage of petroleum or its products. Hydrocarbons which may be produced by the reduction of sapropel in marine sediments may be oxidized by such bacteria *in situ* as rapidly as they are formed, thereby preventing their accumulation in detectable quantities. As a matter of fact, traces of complex compounds such as polyene pigments and their allies are about the only hydrocarbons found in recent sediments with the exception of methane. The abundance of hydrocarbon-oxidizing bacteria in storage tank water suggests that they may play a role in the decomposition of petroleum products. Marine bacteria which oxidize natural and synthetic rubber, $(C_5H_8)_x$, have been described elsewhere by the authors.[5]

It is still indeterminate whether hydrocarbons are utilized in an anærobic environment although there is some evidence that certain substances such as nitrate and possibly sulfate may serve as hydrogen-acceptors in the absence of free oxygen. Low concentrations of hydrocarbons appear to be oxidized quantitatively to carbon dioxide and water, but with larger concentrations, methane and organic acids have been detected as end-products. Adsorbing the hydrocarbons on sand has proved to be a satisfactory method of providing for the dispersion of the hydrocarbons which are virtually insoluble in water thereby rendering them more susceptible to bacterial attack.

[5] ZoBell, C. E., and Grant, C. W., *Science*, 1942.

LIST OF CONTRIBUTIONS

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Number

1. Sverdrup, H. U. On the evaporation from the oceans. *Jour. Marine Research*, v. 1, p. 3-14, 1937.

2. ZoBell, C. E. Studies on the bacterial flora of marine bottom sediments. *Jour. Sed. Petrol.*, v. 8, p. 10-18, 1938.

3. Allen, W. E. Non-visible specimens. *Turtox News*, v. 16, p. 1-3, 1938.

4. Sverdrup, H. U. Notes on erosion by drifting snow and transport of solid material by sea ice. *Amer. Jour. Sci.*, v. 35, p. 370-73, 1938.

5. ZoBell, C. E., and S. C. Rittenberg. The occurrence and characteristics of chitinoclastic bacteria in the sea. *Jour. Bacter.*, v. 35, p. 275-87, 1938.

6. Natland, M. L. New species of foraminifera from the west coast of North America and from later Tertiary of the Los Angeles Basin. *SCRIPPS INSTITUTION BULLETIN*, tech. ser., v. 4, p. 137-64, 1938.

7. LaFond, E. C. Relationship between mean sea level and sand movement. *Science*, v. 88, p. 112-13, 1938.

8. Myers, E. H. The present state of our knowledge of the life history of the foraminifera. *Nat. Acad. Sci., Proc.*, v. 24, p. 10-17. 1938.

9. Sumner, F. B., and P. Doudoroff. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, v. 74, p. 403-29, 1938.

10. Young, R. T. The effect of balanced versus unbalanced solutions of pH and distilled water on fish. *Ecology*, v. 19, p. 126-40, 1938.

11. McEwen, G. F. Modern dynamical oceanography: an achievement of application to ocean observations of principles of mechanics and heat. *Philos. Soc., Proc.*, v. 79, p. 145-66, 1938.

12. Allen, W. E. Quantity collecting of planktonic diatoms. *Science*, v. 87, p. 171-72, 1938.

13. Shepard, F. P., and G. E. MacDonald. Sediments of Santa Monica Bay, California. *A. A. P. G., Bull.*, v. 22, p. 201-16, 1938.

14. Cupp, E. E., and W. E. Allen. Plankton diatoms of the Gulf of California. *Allan Hancock Pacific Exped.*, v. 3. p. 61-99, 1938.

15. Sverdrup, H. U. On the process of upwelling. *Jour. Marine Research*, v. 1, p. 155-64, 1938.

16. ZoBell, C. E., and H. D. Michener. A paradox in the adaptation of marine bacteria to hypotonic solutions. *Science*, v. 87, p. 328-29, 1938.

17. Fox, D. L, and C. R. Moe. An Astacene-like carotenoid from a Pacific Coast anemone, *Epiactis prolifera*. *Nat. Acad. Sci., Proc.*, v. 24, p. 230-35, 1938.

Number

18. Gordon, R. D. Note on estimating bacterial populations by the dilution method. *Nat. Acad. Sci., Proc.*, v. 24, p. 212-15, 1938.

19. Young, R. T. The life history of a trematode (*Levinseniella cruzi?*) from the shore birds (*Limosa fedoa* and *Catoptrophorus semipalmatus inornatus*). *Biol. Bull.*, v. 74, p. 319-39, 1938.

20. Johnson, M. W. Concerning the copepod *Eucalanus elongatus* Dana and its varieties in the northeast Pacific. *SCRIPPS INSTITUTION BULLETIN, tech. ser.*, v. 4, p. 165-80, 1938.

21. Allen, W. E. "Red water" along the west coast of the United States in 1938. *Science*, v. 88, p. 55-56, 1938.

22. Sverdrup, H. U. On the explanation of the oxygen minima and maxima in the oceans. *Jour. du Conseil*, v. 13, p. 163-172, 1938.

23. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1937 to April 1938. *Amer. Geophys. Union, Trans.*, p. 238-42, 1938.

24. Fleming, R. H. Tides and currents in the Gulf of Panama. *Jour. Marine Research*, v. 1, p. 192-206, 1938.

25. McEwen, G. F. Some energy relations between the sea surface and the atmosphere. *Jour. Marine Research*, v. 1, p. 217-38, 1938.

26. Monk, C. R. An aqueous medium for mounting small objects. *Science*, v. 88, p. 174-76, 1938.

27. Evans, R. D., A. F. Kip, E. G. Moberg. The radium and radon content of Pacific Ocean water, life and sediments. *Amer. Jour. Sci.*, v. 36, p. 241-59, 1938.

28. Ritter, W. E. Dr. Fred Baker. *Science*, v. 88, p. 48-49, 1938.

29. Coe, W. R. Influence of association on the sexual phases of gastropods having protandric consecutive sexuity. *Biol. Bull.*, v. 75, p. 274-85, 1938.

30. Marks, G. W. The copper content and copper tolerance of some species of mollusks of the southern California coast. *Biol. Bull.*, v. 75, p. 224-37, 1938.

31. Sumner, F. B., and P. Doudoroff. Some effects of light intensity and shade of background upon the melanin content of Gambusia. *Nat. Acad. Sci.*, v. 24, p. 459-63, 1938.

32. Sumner, F. B., and P. Doudoroff. The effects of light and dark backgrounds upon the incidence of a seemingly infectious disease in fishes. *Nat. Acad. Sci., Proc.*, v. 24, p. 463-66, 1938.

33. Coe, W. R. Sexual phases in the gastropod *Crucibulum spinosum*. *Jour. Morphology*, v. 63, p. 345-61, 1938.

34. Allen, W. E. The Templeton Crocker Expedition to the Gulf of California in 1935—The phytoplankton. *Amer. Microsc. Soc., Trans.*, v. 57, p. 328-35, 1938.

Number

35. ZoBell, C. E. The sequence of events in the fouling of submerged surfaces. *Official Digest, Fed'n Paint and Varnish Prod. Clubs*, no. 178, p. 379-85, 1938.

36. Coe, W. R. A new genus and species of Hoplonemertea having differential polar sexuality. *Zoologischer Anzeiger*, v. 124, p. 220-24, 1938.

37. Gordon, R. D., and C. E. ZoBell. Note on the successive dilution method for estimating bacterial population. *Zentralbl. f. Bakteriol.*, II Abt., Bd. 99, p. 318-20, 1938.

38. Fox, D. L. An illustrated note on the mating and egg-brooding habits of the two-spotted octopus. *San Diego Soc. Nat. Hist., Trans.*, v. 9, p. 31-34, 1938.

39. Shepard, F. P., and C. E. LaFond. Undertow—A discussion. *Science*, v. 89, p. 78-76 1938.

40. ZoBell, C. E., and C. B. Feltham. Bacteria as food for certain marine invertebrates. *Jour. Marine Research*, v. 1, p. 312-27, 1938.

41. ZoBell, C. E., and J. H. Long. Studies on the isolation of bacteria-free cultures of marine phytoplankton. *Jour. Marine Research*, v. 1, p. 328-34, 1938.

42. Doudoroff, Peter. Reactions of marine fishes to temperature gradients. *Biol. Bull.*, v. 75, p. 494-509, 1938.

42-A. Fox, D. L., and M. C. Sargent. Variations in chlorophyll and carotenoid pigments of the brine flagellate, *Dunaliella salina*, induced by environmental concentrations of sodium chloride. *Chem. and Industry*, v. 57, p. 1111, 1938.

43. Allen, W. E. Surface distribution of marine plankton diatoms in the Panama region in 1933. *SCRIPPS INSTITUTION BULLETIN, tech, ser.*, v. 4, p. 181-96, 1939.

44. Emery, K. O. Rapid method of mechanical analysis of sands. *Jour. Sed. Petrol.*, v. 8, p. 105-111, 1938.

45. Sverdrup, H. U. Second note on the logarithmic law of wind structure near the ground. *Royal Meteor. Soc., Quart. Jour.*, v. 65, p. 57-60, 1939.

46. ZoBell, C. E. The biological approach to the preparation of anti-fouling paints. *Sci. Section, Nat'l Paint, Varnish and Lacquer Assoc., Proc.*, Circular 588, p. 149-63, 1939.

47. Sverdrup, H. U. On the influence of stability and instability on the wind profile and the eddy conductivity near the ground. *Fifth Intern. Congr. Applied Mech., Trans.*, p. 369-72, 1939.

48. Sverdrup, H. U. Oceanic circulation. *Idem*, p. 279-293, 1939.

49. Sumner, F. B. Quantitative effects of visual stimuli upon pigmentation. *Amer. Naturalist*, v. 73, p. 219-34, 1939.

50. Sumner, F. B. Human psychology and some things that fishes do. *Scientific Monthly*, v. 49, p. 245-55, 1939.

51. Allen, W. E. Growth of a marine observatory. *Intern. Rev. d. ges. Hydrob. u. Hydrog.*, Bd. 39, p. 464-71, 1939.

Number

51-A. Fox, D. L., and C. F. A. Pantin. The biochemical basis of the colors of the plumose Anemone, *Metridium senile*. *Anat. Record*, v. 75, Suppl., p. 135-36, 1939.

52. Sverdrup, H. U. Physics and geophysics. *Univ. California Faculty Research Lecture*, 23 pp., 1939.

53. Shepard, F. P., R. Revelle, R. S. Dietz. Ocean-bottom currents off the California coast. *Science*, v. 89, p. 488-89, 1939.

54. Gordon, R. D. Some preliminary postulates on stimulus-perception, and consequent probability distribution for behavior. *Psychometrika*, v. 4, p. 89-98, 1939.

55. Coe, W. R. Sexual phases in terrestrial Nemerteans. *Biol. Bull.*, v. 76, p. 416-27, 1939.

56. Allen, W. E. Microcopepoda in marine phytoplankton catches. *Science*, v. 89, p. 532-33, 1939.

57. LaFond, E. C. Variations of sea level on the Pacific Coast of the United States. *Jour. Marine Research*, v. 2, p. 17-29, 1939.

58. Allen, W. E. Cut-throat competition in the sea. *Scientific Monthly*, p. 111-19, 1939.

59. Fleming, R. H. Tables for Sigma-T. *Jour. Marine Research*, v. 2, p. 9-11, 1939.

60. Gordon, R. D. (with foreword by C. E. ZoBell). Estimating bacterial populations by the dilution method. *Biometrika*, p. 167-80, 1939.

61. Young, R. T., Jr., and R. D. Gordon. Report on the penetration of light in the Pacific Ocean off the coast of southern California. *SCRIPPS INSTITUTION BULLETIN, tech. ser.*, v. 4, p. 197-218, 1939.

62. Scheer, B. T. The development of the concept of tissue respiration. *Annals of Science*, v. 4, p. 295-305, 1939.

63. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1938 to April 1939. *Amer. Geophys. Union, Trans.*, p. 422-27, 1939.

64. Johnson, M. W. *Pseudodiaptomus (Pseudodiaptallous) euryhalinus*. A new subgenus and species of Copepoda, with preliminary notes on its ecology. *Amer. Micros. Soc., Trans.*, v. 58, p. 349-55, 1939.

65. Fleming, R. H. The control of diatom populations by grazing. *Cons. Perm. Intern., Jour. du Conseil*, v. 14, p. 210-27, 1939.

66. a. Vaughan, T. W. Presentation of the Agassiz Medal to Harald Ulrik Sverdrup. *Science*, v. 90, p. 24-26, 1939. b. Sverdrup, H. U. Response of the Medallist, *Idem*, p. 26-27, 1939.

67. ZoBell, C. E. Primary film formation by bacteria and fouling. *Collecting Net*, v. 14, 2 pp., 1939.

68. Young, R. T., Jr. Measurements on the transparency of sea-water off the coast of southern California. *Jour. Marine Research*, v. 2, p. 117-125, 1939.

Number

69. Sverdrup, H. U., and W. E. Allen. Distribution of diatoms in relation to the character of water masses and currents off southern California. *Jour. Marine Research*, v. 2, p. 131-44, 1939.

70. Fleming, R. H., and Roger Revelle. Physical processes in the ocean. In: *Recent Marine Sediments, A symposium*, p. 48-141, A.A.P.G., Tulsa, Oklahoma, 1939.

71. Revelle, Roger, and F. P. Shepard. Sediments off the California coast. *Idem*, p. 245-82, 1939.

72. ZoBell, C. E. Occurrence and activity of bacteria in marine sediments. *Idem*, p. 416-27, 1939.

73. Scheer, B. T. "Homing instinct" in salmon. *Quart. Rev. Biol.*, v. 14, p. 408-30, 1939.

74. Allen, W. E. "Phosphorescence" in the sea. *Nautical Gazette*, 2 pp., Aug. 26, 1939.

75. Sverdrup, H. U. Lateral mixing in the deep water of the South Atlantic Ocean. *Jour. Marine Research*, v. 2, p. 195-207, 1939.

76. Jacobs, W. C. A discussion of physical factors governing the distribution of microorganisms in the atmosphere. *Idem*, v. 2, p. 218-24, 1939.

77. Rittenberg, Sydney. Investigations on the microbiology of marine air. *Idem*, v. 2, p. 208-17, 1939.

78. Jacobs, W. C. Sea-level departures on the California coast as related to the dynamics of the atmosphere over the North Pacific Ocean. *Idem*, v. 2, p. 181-94, 1939.

79. Johnson, M. W. The correlation of water movements and dispersal of pelagic larval stages of certain littoral animals, especially the sand crab, *Emerita*. *Idem*, v. 2, p. 236-45, 1939.

80. Coe, W. R. Notes on the morphology and sexuality of the terrestrial Nemertean *Geonemertes palaensis*. *Bishop Museum, Occas. Papers*, p. 205-11, 1940.

81. Sumner, F. B., and M. C. Sargent. Some observations on the physiology of warm spring fishes. *Ecology*, v. 21, p. 45-54, 1940.

82. Sumner, Francis B. Further experiments on the relations between optic stimuli and the increase or decrease of pigment in fishes. *Jour. Exper. Zool.*, v. 83, p. 327-43, 1940.

83. Shepard, Francis P., and Eugene C. LaFond. Sand movements along the Scripps Institution pier. *Amer. Jour. Science*, v. 238, p. 272-85, 1940.

84. Sverdrup, H. U. The currents of the Pacific Ocean and their bearing on the climates of the coasts. *Science*, v. 91, p. 273-82, 1940.

85. Coe, W. R. Revision of the Nemertean fauna of the Pacific coasts of North, Central, and northern South America. *Allan Hancock Pacific Exped.*, v. 2, p. 247-323, 1940. (*Not available for exchange.*)

86. Allen, W. E. Life in a drop of sea water. *Turtox News*, v. 18, no. 4, 3 pp., 1940.

Number

87. Emerson, Ralph, and Denis L. Fox. Gamma-carotene in the sexual phase of the aquatic fungus *Allomyces*. *Roy. Soc. London, Proc., Ser. B, No. 852*, v. 128, p. 275-293, 1940. (*Not available for exchange.*)

88. ZoBell, Claude E., and Janice Stadler. The effect of oxygen tension on the oxygen uptake of lake bacteria. *Jour. Bacteriology*, v. 39, p. 307-22, 1940.

89. ZoBell, Claude E., and Janice Stadler. The oxidation of lignin by lake bacteria. *Archiv. f. Hydrobiol.*, Bd. 37, p. 163-71, 1940. (*Not available for exchange*).

90. Sverdrup, H. U. On the annual and diurnal variation of the evaporation from the ocean. *Jour. Marine Research*, v. 3, p. 93-104, 1940.

91. Sverdrup, H. U. Hydrology, Section 2, Discussion. *B.A.N.Z. Antarctic Research Expedition 1921-31, Repts., Ser. A*, v. 3, *Oceanography*, part 2, *Hydrology*, Sect. 2, p. 88-126, 1940. (*Not available for exchange.*)

92. ZoBell, Claude E., and Jean Conn. Studies on the thermal sensitivity of marine bacteria. *Jour. Bacteriology*, v. 40, p. 223-38, 1940.

93. Lyman, John, and Richard H. Fleming. Composition of sea water. *Jour. Marine Research*, v. 3, p. 134-46, 1940.

94. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1939 to April 1940. *Amer. Geophys. Un., Trans.*, 1940, p. 343-46, 1940.

95. Sverdrup, H. U. Trail blazing in the Pacific. (Research in oceanography). *California Monthly*, p. 10, 11, 28, Dec., 1940. Berkeley.

96. ZoBell, Claude E. Some factors which influence oxygen consumption by bacteria in lake water. *Biol. Bull.*, v. 78, p. 388-402, 1940.

97. Sumner, Francis B. The naturalist as a social phenomenon. *Amer. Naturalist*, v. 47, p. 398-408, 1940.

98. Sumner, Francis B. Quantitative changes in pigmentation, resulting from visual stimuli in fishes and amphibia. *Biol. Reviews*, v. 15, p. 351-75, 1940.

99. Allen, W. E. Discolored sea water. *U. S. Hydrographic Bull.*, no. 2262, 3 pp., 1940.

100. Allen, W. E. Indicator value of phytoplankton at the Scripps Institution. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 529-531, 1940-41.

101. Allen, W. E. Methods of field and laboratory procedure in phytoplankton research at the Scripps Institution of Oceanography. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 525-528, 1940-41.

102. Allen, W. E. Summary of results of twenty years of researches on marine phytoplankton. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 577-583, 1940-41.

103. Fleming, Richard H. Character of the currents off southern California. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 149-160, 1940-41.

Number

104. Fleming, Richard H. Composition of plankton and units for reporting populations and production. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 535-540, 1940-41.

105. Fleming, Richard H. A contribution to the oceanography of the Central American region. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 167-175, 1940-41.

106. Johnson, Martin W. The study of species formation in certain Eucalanus copepods in the North Pacific. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 565-568, 1940-41.

107. La Fond, Eugene C. Sand movements near the beach in relation to tides and waves. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 2, p. 795-799, 1940.

108. Sargent, Marston C. A theoretical definition of production. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 513-516, 1940-41.

109. Sverdrup, H. U. The Gulf of California: Preliminary discussion of the cruise of the *E. W. Scripps* in February and March, 1939. *Sixth Pac. Sci. Congr., California, 1939, Proc*, v. 3, p. 161.166, 1940.41.

110. Sverdrup, H. U. Activities of the Scripps Institution of Oceanography [[1933-1938]]. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 114-123, 1940-41.

111. Shepard, Francis P. Submarine geology and geophysics. *Nature*, v. 146, p. 431-432, 1940.

112. Scheer, Bradley T. Some features of the metabolism of the carotenoid pigments in the California sea mussel (*Mytilus californianus*). *Jour. Biol. Chem.*, v. 136, p. 275-299, 1940.

113. Sverdrup, H. U. The unity of the sciences of the sea. *Sigma Xi Quart.*, v. 28, p. 105-115, 1940.

114. Rittenberg, Sydney C. Bacteriological analysis of some long cores of marine sediments. *Jour. Marine Research*, v. 3, p. 191-201, 1940.

115. ZoBell, Claude E. Effect of oxygen tension on the rate of oxidation of organic matter in sea water by bacteria. *Jour. Marine Research*, v. 3, p. 211-223, 1940.

116. ZoBell, Claude E. The Scripps Institution of Oceanography. *Turtox News*, v. 19, p. 22-23, 1941.

117. Fox, Denis L. Changes in the tissue chloride of the California mussel in response to heterosmotic environments. *Biol. Bull.*, v. 80, p. 111-129, 1941.

118. Shepard, Francis P., and K. O. Emery. Submarine topography off the California coast: Canyons and tectonic interpretations. *Geo . Soc. Amer., Spec. Pap.*, no. 31, 171 pp., 1941. (*Not available for exchange.*)

119. Monk, Cecil R. Marine Harpacticoid copepods from California. *Amer. Microsc. Soc., Trans.*, v. 60, p. 75-99, 1941.

120. McEwen, George F. Observations on temperature, hydrogen-ion concentration, and periods of stagnation and overturning in lakes and reservoirs of San Diego County, California. *SCRIPPS INSTITUTION BULLETIN*, v. 4, no. 9, p. 219-260, 1941.

121. Allen, W. E. Classification and taxonomy. *Turtox News*, v. 19, p. 73-75, 1941.

Number

122. Allen, W. E. Ocean pasturage in California waters. *Scientific Monthly*, v. 52, p. 261-264, 1941.

123. Munk, W. H. Internal waves in the Gulf of California. *Jour. Marine Research*, v. 4, p. 81-91, 1941.

124. ZoBell, Claude E. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *Jour. Marine Research*, v. 4, p. 42-75, 1941.

125. Gordon, Robert D. The estimation of a quotient when the denominator is normally distributed. *Annals of Math. Statistics*, v. 12, p. 115-118, 1941.

126. Shepard, F. P., K. O. Emery, and E. C. La Fond. Rip currents: A process of geological importance. *Jour. Geol.*, v. 49, p. 337-369, 1941.

127. Emery, K. O., and R. H. Tschudy. Transportation of rock by kelp. *Geol. Soc. Amer.*, Bull., v. 52, p. 855-862, 1941.

128. Allen, W. E. Frustules of marine plankton diatoms. *Chronica Botanica*, v. 6, p. 365-367, 1941.

129. Sverdrup, H. U. Water masses and currents of the North Pacific Ocean. *Science*, v. 93, p. 436, 1941.

130. Sverdrup, H. U. An analysis of the ocean currents off the American west coast between 40°N and 40°S. *Univ. Southern California, Chronicle Ser.*, p. 17-20, 1941.

131. Sargent, Marston C. The effect of light intensity on the configuration of the photosynthetic mechanism. *Chronica Botanica*, v. 6, p. 347, 1941.

132. Fox, Denis L., and Bradley T. Scheer. Comparative studies of the pigments of some Pacific Coast echinoderms. *Biol. Bull.*, v. 80, p. 441-455, 1941.

133. Sverdrup, H. U. The influence of bottom topography on ocean currents. p. 66-75 in *Contributions to Applied Mechanics and Related Subjects, Theodore von Kármán Anniversary Volume, Pasadena, California Inst. Technology*, 337 pp., 1941.

134. Fox, D. L., and C. F. A. Pantin. The colours of the plumose anemone *Metridium senile*. *Roy. Soc. London, Trans., Ser. B.*, v. 230, p. 415-45, 1941. (*Not available for exchange.*)

135. Sumner, F. B. Is evolution inscrutable? *Science*, v. 93, p. 521-522, 1941.

136. Sverdrup, H. U., and R. H. Fleming. The waters off the coast of southern California, March to July, 1937. *SCRIPPS INSTITUTION BULLETIN*, v. 4, no. 10, p. 261-378, 1941.

137. Fox, Denis L., and Lloyd J. Anderson. Pigments from marine muds. *Nat. Acad. Sci., Proc.*, v. 27, p. 333-336, 1941.

138. Emery, K. O. Rate of surface retreat of sea cliffs based on dated inscriptions. *Science*, v. 93, p. 617-618, 1941.

139. Emery, K. O. Transportation of rock particles by sea-mammals. *Jour. Sedim. Petrology*, v. 11, p. 92-93, 1941.

Number

140. Allen, W. E. Depth relationships of plankton diatoms in sea water. *Jour. Marine Research*, v. 4, p. 107-111, 1941.

141. Tibby, Richard B. The water masses off the west coast of North America. *Jour. Marine Research*, v. 4, p. 112-121, 1941.

142. Sverdrup, H. U., and Staff. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1940 to April 1941. *Amer. Geophys. Un., Trans., 1941*, p. 490-494, 1941.

143. ZoBell, Claude E. Kelp cutting and its consequences. *California Conservationist*, Sacramento, 3 pp., 1941.

144. Revelle, Roger. Criteria for recognition of sea water in ground-waters. *Amer. Geophys. Un., Trans.*, 1941, p. 593-597, 1941.

145. Coe, Wesley R. Sexual phases in wood-boring mollusks. *Biol. Bull.*, v. 81, p. 168-176, 1941.

146. Shepard, Francis P. Nondepositional physiographic environments off the California coast. *Geol. Soc. Amer. Bull.*, v. 52, p. 1869-1886, 1941.

147. Sverdrup, H. U. The Pacific Ocean. *Science*, v. 94, p. 287-293, 1941.

148. Emery, K. O., and R. S. Dietz. Gravity coring instrument and mechanics of sediment coring. *Geol. Soc. Amer., Bull.*, v. 52, p. 1685-1714, 1941.

149. Gordon, Robert D. Values of Mills' ratio of area to bounding ordinate and of the normal probability integral for large values of the argument. *Annals Math. Statistics*, v. 12, p. 364-366, 1941.

150. ZoBell, Claude E. Apparatus for collecting water samples from different depths for bacteriological analysis. *Jour. Marine Research*, v. 4, p. 173-188, 1941.

151. Allen, Winfred E. Twenty years' statistical studies of marine plankton dinoflagellates of southern California. *Amer. Midland Naturalist*, v. 26, p. 603-635, 1941.

152. ZoBell, Claude E., and Catharine B. Feltham. The bacterial flora of a marine mud flat as an ecological factor. *Ecology*, v. 23, p. 69-78, 1942.

153. Fox, Denis L., and J. F. Danielli. Surface chemistry of carotenoids. I. Astacene. *Biochem. Jour.* (London), v. 35, p. 1388-1395, Dec., 1941.

154. Fox, Denis L., and Sheldon C. Crane. Concerning the pigments of the two-spotted octopus and the opalescent squid. *Biol. Bull.*, v. 82, p. 284-291, 1942.

155. Shepard, Francis P., and Eugene C. La Fond. Mean sea level and sand movement: A reply. *Science*, v. 95, p. 193-194, 1942.

156. Coe, Wesley R. The reproductive organs of the prosobranch mollusk *Crepidula onyx* and their transformation during the change from male to female phase. *Jour. Morphology*, v. 70, p. 501-512, 1942.

157. Sumner, Francis B., and Urless N. Lanham. Studies of the respiratory metabolism of warm and cool spring fishes. *Biol. Bull.*, v. 82, p. 313-27, 1942.

Number

158. Jacobs, Woodrow C. On the energy exchange between sea and atmosphere. *Jour. Marine Research*, v. 5, p. 37-66, 1942.

159. ZoBell, Claude E. Microorganisms in marine air. *A.A.A.S. Publ.* no. 17, p. 55-68, 1942.

160. Dietz, R. S., K. O. Emery, and F. P. Shepard. Phosphorite deposits on the sea floor off southern California. *Geol. Soc. Amer., Bull.*, v. 53, p. 815-848, 1942.

161. Coe, Wesley R., and Denis L. Fox. Biology of the California sea-mussel (*Mytilus californianus*). I. Influence of temperature, food supply, sex, and age on the rate of growth. *Jour. Exper. Zool.*, v. 90, p. 1-30, 1942.

162. Coe, Wesley R. Influence of natural and experimental conditions in determining shape of shell and rate of growth in gastropods of the genus Crepidula. *Jour. Morphology*, v. 71, p. 35-51, 1942.

163. Johnson, Martin W. Some observations on the feeding habits of the octopus. *Science*, v. 95, p. 478-479, 1942.

164. Sverdrup, H. U. Oceanography for meteorologists. *New York, Prentice-Hall.* 246 pp., 1942. (*Not available for exchange.*)

165. Sverdrup, H. U., and Staff. Oceanographic observations on the *E. W. Scripps* cruises of 1938. *SCRIPPS INSTITUTION, RECORDS OF OBSERVATIONS*, v. 1, no. 1, 63 pp., 1942.

166. Johnson, Martin W. Concerning the hitherto unknown males of the copepods *Microsetella rosea* (Dana), *Vettoria granulosa* (Giesbrecht), and *Corissa parva* Farran. *Amer. Micros. Soc.*, Trans., v. 61, p. 430-437, 1942.

167. Fox, Denis L. The carotenoid pigments of cryptogams. *Chronica Botanica*, v. 7, p. 196-198, 1942.

168. Grant, Carroll W. Studies in marine microbiology at the Scripps Institution of Oceanography, University of California, La Jolla. *Amer. Phil. Soc.*, Year Book, 1941, p. 157-158, 1942.

169. ZoBell, Claude E. Bacteria of the marine world. *Scientific Monthly*, v. 55, p. 320-330, 1942.

170. Johnson, Martin W., and Weldon M. Lewis. Pelagic larval stages of the sand crabs *Emerita analoga* (Stimpson), *Blepharipoda occidentalis* Randall, and *Lepidopa myops* Stimpson. *Biol. Bull.*, v. 83, p. 67-87, 1942.

171. Gilbert, J. Y. The errors of the Sedgwick-Rafter counting chamber in the enumeration of phytoplankton. *Amer. Micros. Soc.*, Trans., v. 61, p. 217-226, 1942.

172. Sumner, Francis B. Where does adaptation come in? *Amer. Naturalist*, v. 76, p. 433-444, 1942.

173. ZoBell, Claude E., and Carroll W. Grant. Bacterial activity in dilute nutrient solutions. *Science*, v. 96, p. 189, 1942.

Number

174. Doudoroff, Peter. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.*, v. 83, p. 219-244, 1942.

175. Sverdrup, H. U., and Staff. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1941 to April 1942. *Amer. Geophys. Un.*, Transactions, p. 323-325, 1942.

176. Wohnus, J. Frederick. The kelp resources of southern California. *California Fish and Game*, v. 28, p. 199-205, 1942.

177. Wohnus, J. Frederick. The development of the sporophyte of *Macrocystis pyrifera*. *Turtox News*, v. 20, p. 133-136, 1942.

178. Sverdrup, H. U., M. W. Johnson, and R. H. Fleming. The oceans: Their physics, chemistry, and general biology. *New York, Prentice-Hall.* 1087 pp., 1942. (*Not available for exchange*).

179. ZoBell, Claude E., and Carroll W. Grant. The bacterial oxidation of rubber. *Science*, v. 96, p. 379-380, 1942.

180. Allen, W. E. Occurrences of "red water" near San Diego. *Science*, v. 96, p. 471, 1942.

181. ZoBell, Claude E. Changes produced by microorganisms in sediments after deposition. *Jour. Sedim. Petrol.*, v. 12, p. 127-136, 1942.

182. Grant, Carroll W., and Claude E. ZoBell. Oxidation of hydrocarbons by marine bacteria. *Soc. Exper. Biol. and Medicine, Proc.*, v. 51, p, 266-267, 1942.